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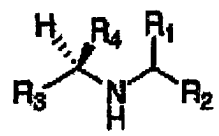

(I)

(57) Abstract: This invention relates to a novel class of compounds, represented by the formula (I) below, wherein the meanings of R_1, R_2, R_3 and R_4 are indicated therein, which are cysteine protease inhibitors, including but not limited to, inhibitors of cathepsins K, L, S and B. These compounds are useful for treating diseases in which inhibition of bone resorption is indicated, such as osteoporosis, osteoarthritis and rheumatoid arthritis.

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TITLE OF THE INVENTION
CATHEPSIN INHIBITORS

FIELD OF THE INVENTION

The invention relates generally to inhibitors of protein activity, and specifically to cathepsin inhibitors.

BACKGROUND OF THE INVENTION

Many cathepsins belong to the papain superfamily of cysteine proteases. These proteases function in the normal physiological as well as pathological degradation of connective tissue. Cathepsins play a major role in intracellular protein degradation and turnover and remodeling. These cathepsins are naturally found in a wide variety of tissues. To date, a number of cathepsin have been identified and sequenced from a number of sources; for example, cathepsin B, F, H, L, K, S, W, and Z have been cloned. Furthermore, the sequence of Cathepsin K can be found in PCT Application WO 96/13523, Khepri Pharmaceuticals, Inc., published May 9, 1996, which is hereby incorporated by reference in its entirety. Cathepsin L is implicated in normal lysosomal proteolysis as well as several diseases states, including, but not limited to, metastasis of melanomas. Cathepsin S is implicated in Alzheimer's disease and certain autoimmune disorders, including, but not limited to juvenile onset diabetes, multiple sclerosis, pemphigus vulgaris, Graves' disease, myasthenia gravis, systemic lupus erythemotasus, rheumatoid arthritis and Hashimoto's thyroiditis; allergic disorders, including, but not limited to asthma; and allogenic immunbe responses, including, but not limited to, rejection of organ transplants or tissue grafts. Increased Cathepsin B levels and redistribution of the enzyme are found in tumors, suggesting a role in tumor invasion and matastasis. In addition, aberrant Cathepsin B activity is implicated in such disease states as rheumatoid arthritis, osteoarthritis, pneumocystisis carinii, acute pancreatitis, inflammatory airway disease and bone and joint disorders.

Cysteine protease inhibitors such as E-64 (*trans*-epoxysuccinyl-L-leucylamide-(4-guanidino) butane) are known to be effective in inhibiting bone resorption. *See* Delaisse, J. M. *et al.*, 1987, *Bone* 8:305-313, which is hereby incorporated by reference in its entirety. Recently, cathepsin K was cloned and found specifically expressed in osteoclasts *See* Tezuka, K. *et al.*, 1994, *J Biol Chem* 269:1106-1109; Shi, G. P. *et al.*,1995, *FEBS Lett* 357:129-134; Bromme, D. and Okamoto, K., 1995, *Biol Chem Hoppe Seyler* 376:379-384; Bromme, D. *et al.*, 1996, *J Biol Chem* 271:2126-2132; Drake, F. H. *et al.*, 1996, *J Biol Chem* 271:12511-12516, which are hereby incorporated by reference in their entirety. Concurrent to the cloning, the autosomal recessive disorder, pycnodysostosis, characterized by an osteopetrotic phenotype with a decrease in bone resorption, was mapped to mutations present in the cathepsin K gene. To date, all mutations identified in the cathepsin K gene are known to result in inactive protein. *See* Gelb, B. D. *et al.*, 1996, *Science* 273:1236-1238; Johnson, M. R. *et al.*, 1996, *Genome Res*

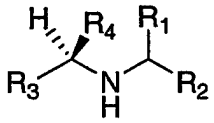
6:1050-1055, which are hereby incorporated by reference in their entirety. Therefore, it appears that cathepsin K is involved in osteoclast mediated bone resorption.

Cathepsin K is synthesized as a 37 kDa pre-pro enzyme, which is localized to the lysosomal compartment and where it is presumably autoactivated to the mature 27 kDa enzyme at low pH. *See* McQueney, M. S. *et al.*, 1997, *J Biol Chem* 272:13955-13960; Littlewood-Evans, A. *et al.*, 1997, *Bone* 20:81-86, which are hereby incorporated by reference in their entirety. Cathepsin K is most closely related to cathepsin S having 56 % sequence identity at the amino acid level. The S_2P_2 substrate specificity of cathepsin K is similar to that of cathepsin S with a preference in the P1 and P2 positions for a positively charged residue such as arginine, and a hydrophobic residue such as phenylalanine or leucine, respectively. *See* Bromme, D. *et al.*, 1996, *J Biol Chem* 271: 2126-2132; Bossard, M. J. *et al.*, 1996, *J Biol Chem* 271:12517-12524, which are hereby incorporated by reference in their entirety. Cathepsin K is active at a broad pH range with significant activity between pH 4-8, thus allowing for good catalytic activity in the resorption lacunae of osteoclasts where the pH is about 4-5.

Human type I collagen, the major collagen in bone is a good substrate for cathepsin K. *See* Kafienah, W., *et al.*, 1998, *Biochem J* 331:727-732, which is hereby incorporated by reference in its entirety. *In vitro* experiments using antisense oligonucleotides to cathepsin K, have shown diminished bone resorption *in vitro*, which is probably due to a reduction in translation of cathepsin K mRNA. *See* Inui, T., *et al.*, 1997, *J Biol Chem* 272:8109-8112, which is hereby incorporated by reference in its entirety. The crystal structure of cathepsin K has been resolved. *See* McGrath, M. E., *et al.*, 1997, *Nat Struct Biol* 4:105-109; Zhao, B., *et al.*, 1997, *Nat Struct Biol* 4: 109-11, which are hereby incorporated by reference in their entirety. Also, selective peptide based inhibitors of cathepsin K have been developed *See* Bromme, D., *et al.*, 1996, *Biochem J* 315:85-89; Thompson, S. K., *et al.*, 1997, *Proc Natl Acad Sci U S A* 94:14249-14254, which are hereby incorporated by reference in their entirety. Accordingly, inhibitors of Cathepsin K can reduce bone resorption. Such inhibitors would be useful in treating disorders involving bone resorption, such as osteoporosis.

SUMMARY OF THE INVENTION

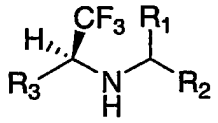
The invention provides compounds that are capable of treating and/or preventing cathepsin dependent conditions or disease states in a mammal in need thereof. The invention provides compounds of the generic formula:



where R_4 is a non-hydrogen substituent with electron-withdrawing character adequate to, in conjunction with R_1, R_2 and R_3, reduce the pKa of the nitrogen to <6 in aqueous media,

where R_1 is a substituent binding in the S_1 subsite of the active site of cathepsins, and where R_2 is a substituent binding in the S_2 subsite of the active site of cathepsins, and where R_3 is a substituent binding in the S_3 subsite of the active site of cathepsins.

More specifically, the invention provides compounds of the formula:



where R_1 is a substituent binding in the S_1 subsite of the active site of cathepsins, and where R_2 is a substituent binding in the S_2 subsite of the active site of cathepsins, and where R_3 is a substituent binding in the S_3 subsite of the active site of cathepsins..

A special nomenclature has evolved for describing the active site of a protease inhibitor. *See*, U.S. Pat. No. 6,333,402, incorporated herein by reference. Starting at the residue on the amino side of the scissile bond of the substrate, and moving away from the bond, residues are named P_1, P_2, P_3, etc. Residues that follow the scissile bond are called P_1', P_2', P_3', etc. It has been found that the main chain of protein inhibitors having very different overall structure are highly similar in the region between P_3 and P_3' with especially high similarity for P_2, P_1 and P_1'. It is generally accepted that each protease active site has subsites S_1, S_2, *etc.* that receive the side groups of residues P_1, P_2, *etc.* of the substrate or inhibitor and subsites S_1', S_2', *etc.* that receive the side groups of P_1', P_2', etc. of the substrate or inhibitor. It is the interactions between the S subsites and the P side groups that give the protease specificity with respect to substrates and the inhibitors specificity with respect to proteases. This nomenclature is generalized to refer to non-peptide inhibitors of proteases where the region of the non-peptide inhibitor that interacts with protease subsites S_1, S_2, *etc.*, would be referred to as P_1, P_2, *etc.*, respectively.

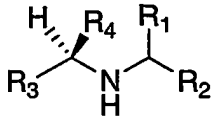
The P_2-P_3 amide bond in dipeptidic cathepsin inhibitors may be replaced by a substituted ethylamine moeity. The electron-withdrawing properties of the R_1, R_2, R_3, and R_4 groups together render the P2 amine non-basic at physiological pH. This fragment is thus capable of making an important neutral hydrogen bond to a cathepsin glycine conserved amongst the entire papain family of cathepsins.

Both amides and anilines are capable of making hydrogen bonds to carbonyl groups, but have metabolic liabilities. By itself, the ethylamine is basic and is protonated in biological systems, generating a charge that reduces binding to the target enzyme. With sufficiently electron-withdrawing R_4 (or more specifically trifluoroethylamine), the amine is not protonated and provides a superior hydrogen bond to acceptor oxygen in the cathepsin active site. In cathepsins, an important acceptor oxygen is situated in between the S_2 and S_3 subsites of the active site; in cathepsin K this is Gly66. This residue is a conserved residue amongst this entire papain family of proteins, including cathepsins B, F, H, K, L, L2, O, S, W, and Z, falcipain, falcipain-1 and falcipain 2. Thus, using the non-basic ethylamine linker to join

inhibitor fragments binding the S_2 and S_3 subsites of cathepsins allows for more potent inhibition than the corresponding amides.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a composition of matter having the chemical formula:



and having no more than 70 nonhydrogen atoms each independently selected from C, O, N, S, P, F, Cl, Br or I;

wherein R_4 is a non-hydrogen electron-withdrawing substituent such that, together with R_1, R_2 and R_3, the basicity of the nitrogen is lowered to less than a pKa of 6;

wherein a molecule of the composition is interacting with a cathepsin such that the CH-NH region in the chemical formula is interacting favorably with the cathepsin between S_2 and S_3, R_1 interacts favorably with S_1 but not S_3 of the cathepsin active site, R_2 interacts favorably with S_2 but not S_3 of the cathepsin active site, and R_3 interacts favorably with S_3 but not S_2 or S_1 of the cathepsin active site.

In a class of the invention the cathepsin is selected from cathepsin B, F, H, K, L, L_2, O, S, W and Z. In a subclass of the invention, the cathepsin is selected from cathepsin K, L, S, and B. In a further subclass of the invention, the cathepsin is cathepsin L. In a further subclass of the invention, the cathepsin is cathepsin S. In a further subclass of the invention, the cathepsin is cathepsin B. In a further subclass of the invention, the cathepsin is cathepsin F.

In a class of the invention, R_4 does not interact favorably with subsites S_2, S_3 and S_1, respectively, of a cathepsin active site. In another class of the invention, R_4 is selected from $-CF_3$, $-CHF_2$, $-CH_2F$, $-CF_2R_5$, and $-CHF\,R_5$, wherein R_5 is C_{1-6} alkyl, aryl, or heteroaryl optionally substituted with 1 to 4 substituents selected from halo, C_{1-3} alkyl, C_{1-3} alkoxy, hydroxy, hydroxyalkyl, keto, cyano, heterocyclyl, C_{3-8} cycloalkyl, SO_mC_{1-3} alkyl , NH_2, NO_2 or $O(C{=}O)C_{1-3}$ alkyl, wherein m is an integer from zero to two.

In a class of the invention, R_2 has at least one carbon or sulfur atom which simultaneously fulfills the following three distance critieria: it is within 7 Å of $C\alpha_{26}$, and it is within 8.5 Å of $C\alpha_{68}$ and it is within 7 Å of $C\alpha_{134}$ of a cathepsin. In another class of the invention, R_2 comprises nonpolar regions. In another class of the invention, R_2 comprises lipophilic regions.

In a class of the invention, R_1 comprises a region that stably fits into subsite S_1 of a cathepsin active site, having at least one carbon atom within 5 Å of $C\alpha_{25}$ of a cathepsin. In another class of the invention, R_1 is non-immunogenic

In a class of the invention, R_3 has at least one carbon or sulfur atom which simultaneously fulfills the following two distance critieria: it is within 5.5 Å of $C\alpha_{66}$, and it is within 7 Å of $C\alpha_{60}$ of a cathepsin. In another class of the invention, R_3 comprises nonpolar regions. In another class of the invention, R_3 comprises lipophilic regions.

In a class of the invention, the nitrogen has a pKa of less than 6 and makes a hydrogen bond with the cathepsin amide carbonyl of glycine 66 of a cathepsin.

In a class of the invention, the compound has a molecular weight of less than 1000 daltons. In a class of the invention, the compound forms a covalent bond with cysteine 25 of a cathepsin. In a class of the invention, the compound binds to the active site of a cathepsin with an IC_{50} of less than 10 micromolar in a purified enzyme assay.

In a class of the invention, the pKa of the nitrogen of the secondary amine shown in claim 1 is <5 in an aqueous medium.

The term "lipophilic," as the term is used herein, refers to a compound, which, as a separate entity, is more soluble in nonpolar solvents (e.g. cyclohexane) than water. The term "lipophilic group", in the context of being attached to a molecule, refers to a region of the molecule having high hydrocarbon content thereby giving the group high affinity to nonpolar solvents or lipid phases. A lipophilic group can be, for example, an alkyl or cycloalkyl chain(preferably n-alkyl) of less than 30 carbons. To further illustrate, lipophilic groups include the alkyl chains attached to naturally-occurring and synthetic aromatic and non-aromatic moieties such as fatty acids, esters and alcohols, and other lipid molecules. Other examples of lipophilic molecules are cage structures such as adamantane and buckminsterfullerenes, and aromatic hydrocarbons such as benzene, perylene, phenanthrene, anthracene, naphthalene, pyrene, chrysene, and naphthacene. Specifically included in the term "lipophilic group" as used herein are the carbons and attached hydrogens of alkyl chains, cycloalkyl chains, aryl rings, or heteroaryl rings. The term "lipophilic group" as used herein also includes divalent sulfur.

The term "substantially homologous", when used in connection with amino acid sequences, refers to sequences which are substantially identical to or similar in sequence, giving rise to a homology in conformation and thus to similar biological activity. The term is not intended to imply a common evolution of the sequences. Typically, "substantially homologous" sequences are at least 50% more preferably at least 80% identical in sequence, at least over any regions known to be involved in the desired activity. Most preferably, no more than five residues, other than at the termini, are different. Preferably, the divergence in sequence, at least in the aforementioned regions, is in the form of "conservative modifications".

"Conservative modifications" are defined as (a) conservative substitutions of amino acids as hereafter defined; and (b) single or multiple insertions or deletions of amino acids at the termini, at interdomain boundaries, in loops or in other segments of relatively high mobility (as indicated, e.g., by the failure to clearly resolve their structure upon X-ray diffraction analysis or NMR). Preferably, except

at the termini, no more than about five amino acids are inserted or deleted at a particular locus, and the modifications are outside regions known to contain binding sites important to activity.

Conservative substitutions are herein defined as exchanges within one of the following five groups: (1) small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr (Pro, Gly); (2) polar, negatively charged residues: and their amides Asp, Asn, Glu, Gln; (3) polar, positively charged residues: His, Arg, Lys; (4) large, aliphatic, nonpolar residues: Met, Leu, Ile, Val (Cys); and (5) large, aromatic residues: Phe, Tyr, Trp. Residues Pro, Gly and Cys are parenthesized because they have special conformational roles. Cys participates in formation of disulfide bonds. Gly imparts flexibility to the chain. Pro imparts rigidity to the chain and disrupts alpha helices. These residues may be essential in certain regions of the polypeptide, but substitutable elsewhere. Semi-conservative substitutions are defined to be exchanges between two of groups (1)-(5) above which are limited to supergroup (a), comprising (1), (2) and (3) above, or to supergroup (b), comprising (4) and (5) above.

The term "cathepsin" or "cathepsins" as used herein refers to enzymes belonging to the papain family, i.e. where the active form of the enzyme is folded similarly to papain (Conserved Domain smart00645.7 in the NCBI Conserved Domain Database http: //www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=15045). Only the cathepsins in human, mouse, rabbit, primates, rat, and *plasmodium falciparum* are being referred to herein. The human cathepsins specifically included are B, F, H, K, L, L2, O, S, W, and Z; also specifically included are enzymes in mouse, rabbit, primates, and rat exhibiting greater than 80% sequence identity (comparing the active forms of the enzymes) to the most similar of the preceding human cathepsins. Also specifically included are falcipain, falcipain-1 and falcipain-2 from *plasmodium falciparum*, and any other enzymes from *plasmodium falciparum* exhibiting greater than 80% sequence identity to the most similar of these, again comparing the active forms of the enzymes.

A specific amino acid residue(e.g. glycine 66) or a Cα (e.g. $C\alpha_{66}$) in a cathepsin is referred to herein *via* a combination of the residue numbering used for the active form of Cathepsin K and the primary sequence alignment given in Figure 1. This sequence alignment is for the relevant portion of the active form of the protein for human cathepsins and falcipains as labeled in the Figure. In the Figure, some of the key residue numbers referred to herein are given, formatted vertically above the aligned sequences. Other residue numbers referred to can found by counting along from the closest numbered residue or by direct reference to the Cathepsin K sequence. The numbering is obtained based on the SWISSPROT primary accession #P43235 for human cathepsin K, where the first residue in the CHAIN, the active form of the protein (residue number 115 in #P43235) is reset herein to be residue number 1. The sequence alignment herein is used to generalize the reference to a Cathepsin K residue to the other human cathepsins and the falcipains. The reference to residues in mouse, rabbit, primates, and rat cathepsins is implied by a standard primary sequence alignment, using default parameters, to the most highly homologous sequence specified below:

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                          22
                          56
                          **
CathepsinK : APDSVDYRKKG----YVTPVKNQGQ---CGSCWAFSSVGALEGQLKK-KT
CathepsinB : LPASFDAREQWPQCPTIKEIRDQGS---CGSCWAFGAVEAISDRICIHTN
CathepsinF : APPEWDWRSKG----AVTKVKDQGM---CGSCWAFSVTGNVEGQWFL-NQ
CathepsinH : YPPSVDWRKKGN---FVSPVKNQGA---CGSCWTFSTTGALESAIAI-AT
CathepsinL : APRSVDWREKG----YVTPVKNQGQ---CGSCWAFSATGALEGQMFR-KT
CathepsinO : LPLRFDWRDKQ----VVTQVRNQQM---CGGCWAFSVVGAVESAYAI-KG
CathepsinS : LPDSVDWREKG----CVTEVKYQGS---CGACWAFSAVGALEAQLKL-KT
CathepsinW : VPFSCDWRKVAG---AISPIKDQKN---CNCCWAMAAAGNIETLWRI-SF
CathepsinZ : LPKSWDWRNVDGVN-YASITRNQHIPQYCGSCWAHASTSAMADRINIKRK
CathepsinL2: LPKSVDWRKKG----YVTPVKNQKQ---CGSCWAFSATGALEGQMFR-KT
Falcipain  : VPEILDYREKG----IVHEPKDQGL---CGSCWAFASVGNIESVFAK-KN
Falcipain2 : DRIAYDWRLHG----GVTPVKDQAL---CGSCWAFSSVGSVESQYAI-RK
Falcipain3 : DHAAYDWRLHS----GVTPVKDQKN---CGSCWAFSSIGSVESQYAI-RK

                          6  667
                          0  680
                          *  ***
CathepsinK : GK--LLNLSPQNLVDCV---SENDGCGGGYMTNAFQYVQKNR-GIDSEDA
CathepsinB : AH-VSVEVSAEDLLTCCGS-MCGDGCNGGYPAEAWNFWTRKG--LVSGGL
CathepsinF : GT--LLSLSEQELLDCD---KMDKACMGGLPSNAYSAIKNLG-GLETEDD
CathepsinH : GK--MLSLAEQQLVDCAQD-FNNHGCQGGLPSQAFEYILYNK-GIMGEDT
CathepsinL : GR--LISLSEQNLVDCSGP-QGNEGCNGGLMDYAFQYVQDNG-GLDSEES
CathepsinO : KP--LEDLSVQQVIDCS---YNNYGCNGGSTLNALNWLNKMQVKLVKDSE
CathepsinS : GK--LVTLSAQNLVDCSTEKYGNKGCNGGFMTTAFQYIIDNK-GIDSDAS
CathepsinW : WD--FVDVSVHELLDCG---RCGDGCHGGFVWDAFITVLNNS-GLASEKD
CathepsinZ : GAWPSTLLSVQNVIDC----GNAGSCEGGNDLSVWDYAHQHG--IPDETC
CathepsinL2: GK--LVSLSEQNLVDCSRP-QGNQGCNGGFMARAFQYVKENG-GLDSEES
Falcipain  : KN--ILSFSEQEVVDCS---KDNFGCDGGCHPFYSFLYVLQN--ELCLGDE
Falcipain2 : KA--LFLFSEQELVDCS---VKNNGCYGGYITNAFDDMIDLG-GLCSQDD
Falcipain3 : NK--LITLSEQELVDCS---FKNYGCNGGLINNAFEDMIELG-GICPDGD
```

CathepsinK : YPYVG--------QEESCMYNPT----------GKAAKCR
CathepsinB : YESHVGCRPYSIPPCEHHVNGSRPPCTGEGDTPKCSKICEPGYSPTYKQD
CathepsinF : YSYQG--------HMQSCNFSAE----------KAKVYIN
CathepsinH : YPYQG--------KDGYCKFQPG----------KAIGFVK
CathepsinL : YPYEA--------TEESCKYNPK----------YSVANDT
CathepsinO : YPFKA--------QNGLCHYFSG----------SHSGFSI
CathepsinS : YPYKA--------MDQKCQYDSK----------YRAATCS
CathepsinW : YPFQGK-------VRAHRCHPKKY----------QKVAWIQ
CathepsinZ : NNYQAKDQECDKFNQCGTCNEFKE----------CHAIRNY
CathepsinL2: YPYVA--------VDEICKYRPE----------NSVANDT
Falcipain : YKYKAK--------DDMFCLNYRC----------KRKVSLS
Falcipain2 : YPYVSN--------LPETCNLKRC----------NERYTIK
Falcipain3 : YPYVSD--------APNLCNIDRC----------TEKYGIK

1
3
4
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CathepsinK : GYREIPEG----NEKALKRAVARVGPVSVAIDASLTSFQFYSKGVYYDES
CathepsinB : KHYGYNSYSVSNSEKDIMAEIYKNGPVEGAFSVYSD-FLLYKSGVYQHVT
CathepsinF : DSVELSQ-----NEQKLAAWLAKRGPISVAINAFGMQFYRHGISRPLRPL
CathepsinH : DVANITIY----DEEAMVEAVALYNPVSFAFEVTQD-FMMYRTGIYSSTS
CathepsinL : GFVDIPK-----QEKALMKAVATVGPISVAIDAGHESFLFYKEGIYFEPD
CathepsinO : KGYSAYDFSD--QEDEMAKALLTFGPLVVIVDAVSWQDYLGGIIQHHCSS
CathepsinS : KYTELPYG----REDVLKEAVANKGPVSVGVDARHPSFFLYRSGVYYEPS
CathepsinW : DFIMLQN-----NEHRIAQYLATYGPITVTINMKPLQLYRKGVIKATPTT
CathepsinZ : TLWRVGDYGSLSGREKMMAEIYANGPISCGIMATER-LANYTGGIYAEYQ
CathepsinL2: GFTVVAPG----KEKALMKAVATVGPISVAMDAGHSSFQFYKSGIYFEPD
Falcipain : SIGAVKE-------NQLILALNEVGPLSVNVGVNND-FVAYSEGVYNGTC
Falcipain2 : SYVSIPD------D-KFKEALRYLGPISISIAASDD-FAFYRGGFYDGEC
Falcipain3 : NYLSVPD------N-KLKEALRFLGPISISVAVSDD-FAFYKEGIFDGEC

CathepsinK : CNS-----DNLNHAVLAVGYGIQKG----------
CathepsinB : GEMMG------GHAIRILGWGVENG----------
CathepsinF : CSP-----WLIDHAVLLVGYGNRSD----------
CathepsinH : CHKTP---DKVNHAVLAVGYGEKNG----------
CathepsinL : CSS-----EDMDHGVLVVGYGFESTE----------SDN
CathepsinO : GE--------ANHAVLITGFDKTGS----------
CathepsinS : CT------QNVNHGVLVVGYGDLNG----------
CathepsinW : CDP-----QLVDHSVLLVGFGSVKSEEGIWAETVSS-----QSQPQPPHP
CathepsinZ : DTT------YINHVVSVAGWGISDG----------
CathepsinL2: CSS-----KNLDHGVLVVGYGFEGAN----------SNN
Falcipain : SEE-------LNHSVLLVGYGQVEKTKLNYNNKIQTYNTKENSNQPDDNI
Falcipain2 : GA------A-PNHAVILVGYGMKDIYNEDTG----------RMEK
Falcipain3 : GD------E-LNHAVMLVGFGMKEIVNPLTK----------KGEK

2
0
9
*

CathepsinK : NKHWIIKNSWGENWGNKGYILMARNKN-------NACGIANLASFPKM--
CathepsinB : TPYWLVANSWNTDWGDNGFFKILRGQD--------HCGIESEVVAGIPRT
CathepsinF : VPFWAIKNSWGTDWGEKGYYYLHRGSG--------ACGVNTMASSAVVD-
CathepsinH : IPYWIVKNSWGPQWGMNGYFLIERGK--------NMCGLAACASYPIPLV
CathepsinL : NKYWLVKNSWGEEWGMGGYVKMAKDRR-------NHCGIASAASYPTV--
CathepsinO : TPYWIVRNSWGSSWGVDGYAHVKMGSN--------VCGIADSVSSIFV--
CathepsinS : KEYWLVKNSWGHNFGEEGYIRMARNKG-------NHCGIASFPSYPEI--
CathepsinW : TPYWILKNSWGAQWGEKGYFRLHRGSN--------TCGITKFPLTARVQK
CathepsinZ : TEYWIVRNSWGEPWGERGWLRIVTSTYKDGKGARYNLAIEEHCTFGDPIV
CathepsinL2: SKYWLVKNSWGPEWGSNGYVKIAKDKN-------NHCGIATAASYPNV--
Falcipain : IYYWIIKNSWSKKWGENGFMRLSRNKNGD----NVFCGIGEEVFYPIL--
Falcipain2 : FYYYIIKNSWGSDWGEGGYINLETDENGY----KKTCSIGTEAYVPLLE-
Falcipain3 : HYYYIIKNSWGQQWGERGFINIETDESGL----MRKCGLGTDAFIPLIE-

CathepsinK : ----------(SEQ ID NO: 1)
CathepsinB : D----------(SEQ ID NO: 2)
CathepsinF : ----------(SEQ ID NO: 3)
CathepsinH : ----------(SEQ ID NO: 4)
CathepsinL : ----------(SEQ ID NO: 5)
CathepsinO : ----------(SEQ ID NO: 6)
CathepsinS : ----------(SEQ ID NO: 7)
CathepsinW : PDMKPRVSCPP-(SEQ ID NO: 8)
CathepsinZ : ----------(SEQ ID NO: 9)
CathepsinL2: ----------(SEQ ID NO: 10)
Falcipain : ----------(SEQ ID NO: 11)
Falcipain2 : ----------(SEQ ID NO: 12)
Falcipain3 : ----------(SEQ ID NO: 13)

Figure 1.

Definitions: The term "favorably", as used herein in the term "interacts favorably", refers to the favorable result of a molecular modeling calculation in which a computer model of the ligand molecule is placed into the active site of a computer model of the cathepsin, making a ligand:cathepsin complex, and the ligand:cathepsin complex is energy minimized using a standard molecular mechanics forcefield such as MMFF94s (T. A. Halgren, *Journal of Computational Chemistry* (1999), **20**, pp. 720-729.). The initial placement of the ligand into the active site, also referred to as "docking", is done so as to optimally allow the ligand:enzyme interactions between the substituents of the ligand and the subsites of the enzyme as stated herein. The computer model of the active site is based on an Xray crystal structure (e.g. Protein Databank entry 1MEM for cathepsin K) if available for the cathepsin of interest (minus the non-protein atoms), or if an Xray crystal structure is not available, one can use a homology-built structure based on the most similar cathepsin for which an Xray structure is available, or simply use directly the most similar cathepsin for which an Xray structure is available. At the start of the energy minimization, no protein atoms have been adjusted to accommodate the ligand, but during the energy minimization, entire protein sidechains having at least one atom within 6 Å of the ligand are allowed to move with the energy minimization. No mainchain atoms of the enzyme are allowed to move during the energy minimization. A continuum dielectric solvent of water may be used, but otherwise default parameters and behaviour of a standard molecular mechanics energy minimization similar to those in Schrodinger Inc.'s software program MacroModel is assumed . A "favorable result of

a modeling calculation" means that after the energy minimization is complete there are no bad steric interactions between the ligand and the active site and there are energy-stabilising hydrogen bonding and lipophilic interactions between the ligand and the active site. If it is believed that the ligand forms a covalent bond with the active site sulfur of cysteine-25, the energy minimization may include that covalent bond in the modeled ligand:cathepsin complex submitted to the calculation. Interactions between the ligand and the cathepsin as claimed herein are based on the geometry of the ligand:cathepsin complex that would result from such an energy minimization.

In one embodiment of the invention, favorable interactions between a substituent of the ligand and S_2 require having at least one carbon or divalent sulfur atom of the substituent simultaneously fulfilling the following three distances: it is within 7 Å of $C\alpha_{26}$, and it is within 8.5 Å of $C\alpha_{68}$ and it is within 7 Å of $C\alpha_{134}$ of the cathepsin. In another embodiment of the invention, favorable interactions between a substituent of the ligand and S_2 require having at least one atom of a lipophilic group of the substituent within 5.5 Å of atoms of two of residues 67, 68, 134. In a further embodiment of the invention relating only to cathepsin B, favorable interactions between a substituent of the ligand and S_2 require having hydrogen bonding between the substituent and glutamate 209 of cathepsin B.

In one embodiment of the invention, favorable interactions between a substituent of the ligand and S_3 require having at least one carbon or divalent sulfur atom of the substituent simultaneously fulfilling the following two distances: it is within 5.5 Å of $C\alpha_{66}$, and it is within 7 Å of $C\alpha_{60}$ of the cathepsin. In another embodiment of the invention, favorable interactions between a substituent of the ligand and S_3 require having at least one atom of a lipophilic group of the substituent within 5.5 Å of $C\alpha_{66}$ and atoms of either residue 60 or 61. In another embodiment of the invention, favorable interactions between a substituent of the ligand and S_3 require having at least one atom of a lipophilic group of the substituent within 5.5 Å of either residue 60 or 61.

In one embodiment of the invention, favorable interactions between a substituent of the ligand and S_1 require having at least one carbon atom of the substituent within 5 Å of $C\alpha_{25}$. In another embodiment of the invention, favorable interactions between a substituent of the ligand and S_1 require having a covalent bond between the active site cysteine sulfur of residue 25 and an electrophilic carbon of the ligand, preferably a carbonyl or nitrile carbon.

In one embodiment of the invention, favorable interactions between the CH-NH region in the chemical formula of the ligand and the cathepsin between S2 and S3 requires having the N of the chemical formula within 4 Å of the peptide oxygen of Gly66, and the C of the chemical formula (referring only the C directly connected with R_4) within 5.5 Å of $C\alpha_{66}$. In another embodiment of the invention, favorable interactions between the CH-NH region in the chemical formula of the ligand and the cathepsin between S2 and S3 requires having the NH of the chemical formula hydrogen bonding to the amide O of residue 66.

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having one to ten carbon atoms unless otherwise specified. For example, C_1-C_{10}, as in "C_1-C_{10} alkyl" is defined to include groups having 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbons in a linear, branched, or cyclic arrangement. For example, "C_1-C_{10} alkyl" specifically includes methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, and so on.

"Alkoxy" or "alkyloxy" represents an alkyl group as defined above, unless otherwise indicated, wherein said alkyl group is attached through an oxygen bridge.

The term "cycloalkyl" or "carbocycle" shall mean cyclic rings of alkanes of three to eight total carbon atoms, unless otherwise indicated, or any number within this range (i.e., cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclooctyl).

As used herein, "aryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 12 atoms in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl, phenanthryl, anthryl or acenaphthyl. In cases where the aryl substituent is bicyclic and one ring is non-aromatic, it is understood that attachment is via the aromatic ring.

The term "heteroaryl", as used herein, represents a stable monocyclic, bicyclic or tricyclic ring of up to 10 atoms in each ring, wherein at least one ring is aromatic and contains from 1 to 4 heteroatoms selected from the group consisting of O, N and S. Heteroaryl groups within the scope of this definition include but are not limited to: benzoimidazolyl, benzofuranyl, benzofurazanyl, benzopyrazolyl, benzotriazolyl, benzothiophenyl, benzoxazolyl, carbazolyl, carbolinyl, cinnolinyl, furanyl, indolinyl, indolyl, indolazinyl, indazolyl, isobenzofuranyl, isoindolyl, isoquinolyl, isothiazolyl, isoxazolyl, naphthpyridinyl, oxadiazolyl, oxazolyl, oxazoline, isoxazoline, pyranyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridopyridinyl, pyridyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinolyl, quinoxalinyl, tetrazolyl, tetrazolopyridyl, thiadiazolyl, thiazolyl, thienyl, triazolyl, dihydrobenzoimidazolyl, dihydrobenzofuranyl, dihydrobenzothiophenyl, dihydrobenzoxazolyl, dihydroindolyl, dihydroquinolinyl, methylenedioxybenzene, benzothiazolyl, benzothienyl, quinolinyl, isoquinolinyl, oxazolyl, and tetra-hydroquinoline. In cases where the heteroaryl substituent is bicyclic and one ring is non-aromatic or contains no heteroatoms, it is understood that attachment is via the aromatic ring or via the heteroatom containing ring, respectively. If the heteroaryl contains nitrogen atoms, it is understood that the corresponding N-oxides thereof are also encompassed by this definition.

As appreciated by those of skill in the art, "halo" or "halogen" as used herein is intended to include chloro, fluoro, bromo and iodo. The term "keto" means carbonyl (C=O). The term "alkoxy" as used herein means an alkyl portion, where alkyl is as defined above, connected to the remainder of the molecule via an oxygen atom. Examples of alkoxy include methoxy, ethoxy and the like.

The term "hydroxyalkyl" means a linear monovalent hydrocarbon raidcal of one to six carbon atoms or a branched monovalent hydrocarbon radical of three to six carbons substituted with one

or two hydroxy groups, provided that if two hydroxy groups are present they are not both on the same carbon atom. Representative examples include, but are not limited to, hydroxymethyl, 2-hydroxyethyl, 2-hydroxypropyl, 3- hydroxypropyl, and the like.

The term "heterocycle" or "heterocyclyl" as used herein is intended to mean a 5- to 10-membered nonaromatic ring, unless otherwise specified, containing from 1 to 4 heteroatoms selected from the group consisting of O, N, S, SO, or SO_2 and includes bicyclic groups. "Heterocyclyl" therefore includes, but is not limited to the following: piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, tetrahydropyranyl, dihydropiperidinyl, tetrahydrothiophenyl and the like. If the heterocycle contains a nitrogen, it is understood that the corresponding N-oxides thereof are also emcompassed by this definition.

The present invention also includes N-oxide derivatives and protected derivatives of compounds of Formula I. For example, when compounds of Formula I contain an oxidizable nitrogen atom, the nitrogen atom can beconverted to an N-oxide by methods well known in the art. Also whencompounds of Formula I contain groups such as hydroxy, carboxy, thiol or anygroup containing a nitrogen atom(s), these groups can be protected with a suitable protecting groups. A comprehensive list of suitable protective groups can be found in T.W. Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, Inc. 1981, the disclosure of which is incorporated herein by reference in its entirety. The protected derivatives of compounds of Formula I can be prepared by methods well known in the art. .

Also included within the scope of the present invention is a pharmaceutical composition which is comprised of a compound as described above and a pharmaceutically acceptable carrier. The invention is also contemplated to encompass a pharmaceutical composition, which is comprised of a pharmaceutically acceptable carrier and any of the compounds specifically disclosed in the present application. These and other aspects of the invention will be apparent from the teachings contained herein.

The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed inorganic or organic acids. For example, conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like, as well as salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like. The preparation of the pharmaceutically acceptable salts described above and other typical pharmaceutically acceptable salts is more fully described by Berg *et al.*, "Pharmaceutical Salts," *J. Pharm. Sci.*, 1977:66:1-19, hereby incorporated by reference. The pharmaceutically acceptable salts of the compounds of this invention can be synthesized from the compounds of this invention which contain a basic or acidic moiety by conventional chemical methods. Generally, the salts of the basic compounds are prepared

either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents. Similarly, the salts of the acidic compounds are formed by reactions with the appropriate inorganic or organic base.

The compounds of the present invention are inhibitors of cathepsins and are therefore useful to treat or prevent cathepsin dependent diseases or conditions in mammals, preferably humans. Specifically, the compounds of the present invention are inhibitors of Cathepsin K and are therefore useful to treat or prevent Cathepsin K dependent diseases or conditions in mammals, preferably humans.

"Cathepsin dependent diseases or conditions" refers to pathologic conditions that depend on the activity of one or more cathepsins. "Cathepsin K dependent diseases or conditions" refers to pathologic conditions that depend on the activity of Cathepsin K. Diseases associated with Cathepsin K activities include osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures, rheumatoid arthritis, osteoarthritis, periprosthetic osteolysis, osteogenesis imperfecta, obesity, atherosclerosis, chronic obstructive pulmonary disease, juvenile onset diabetes, multiple sclerosis, pemphigus vulgaris, Graves' disease, myasthenia gravis, systemic lupus erythemotasus, rheumatoid arthritis and Hashimoto's thyroiditis, asthma, allogenic immune responses, parasitic infection, cancer, metastatic bone disease, hypercalcemia of malignancy or multiple myeloma. In treating such conditions with the instantly claimed compounds, the required therapeutic amount will vary according to the specific disease and is readily ascertainable by those skilled in the art. Although both treatment and prevention are contemplated by the scope of the invention, the treatment of these conditions is the preferred use.

An embodiment of the invention is a method of inhibiting cathepsin activity in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of any of the compounds or any of the pharmaceutical compositions described above.

A class of the embodiment is the method wherein the cathepsin activity is cathepsin K activity.

Another embodiment of the invention is a method of treating or preventing cathepsin dependent conditions in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of any of the compounds or any of the pharmaceutical compositions described above.

A class of the embodiment is the method wherein the cathepsin activity is cathepsin K activity.

Another embodiment of the invention is a method of inhibiting bone loss in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of any of the compounds or any of the pharmaceutical compositions described above. Another embodiment of the invention is a method of reducing bone loss in a mammal in need thereof, comprising administering to

the mammal a therapeutically effective amount of any of the compounds or any of the pharmaceutical compositions described above. The utility of cathepsin K inhibitors in the inhibition of bone resorption is known in the literature. See Stroup G.B. *et al.*, "Potent and selective inhibition of human cathepsin K leads to inhibition of bone resorption in vivo in a nonhuman primate." *J. Bone Miner. Res.*, 16:1739-1746;2001; and Votta, B.J. *et al.*, "Peptide aldehyde inhibitors of cathepsin K inhibit bone resorption both *in vivo* and *in vitro*." *J. Bone Miner. Res.* 12:1396-1406; 1997.

Another embodiment of the invention is a method of treating or preventing osteoporosis in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of any of the compounds or any of the above pharmaceutical compositions described above. The utility of cathepsin K inhibitors in the treatment or prevention of osteoporosis is known in the literature. See, Saftig P. *et al.*, "Impaired osteoclast bone resorption leads to osteoporosis in cathepsin K-deficient mice." *Proc. Natl. Acad. Sci. USA* 95:13453-13458; 1998.

Another embodiment of the invention is a method of treating or preventing rheumatoid arthritic condition in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of any of the compounds or any of the pharmaceutical compositions described above. It is known in the literature that progressive destruction of the periarticular bone is a major cause of joint dysfunction and disability in patients with rheumatoid arthritis (RA). See Goldring SR, "Pathogenesis of bone erosions in rheumatoid arthritis". *Curr. Opin. Rheumatol.* 14: 406-10, 2002. Analysis of joint tissues from patients with RA have provided evidence that cathepsin K positive osteoclasts are the cell types that mediate the focal bone resorption associated with rheumatoid synovial lesion. See Hou, W-S *et al.*, "Comparision of Cathepsin K and S expression within the Rheumatoid and Osteoarthritic Synovium", *Arthritis Rheumatism* 46: 663-74, 2002. In addition, generalized bone loss is a major cause of morbility associated with severe RA. The frequency of hip and spinal fractures is substantially increased in patients with chronic RA. See, Gould *et al*, "Osteoclastic activation is the principal mechanism leading to secondary osteoporosis in rheumatoid arthritis". *J. Rheumatol.* 25: 1282-9, 1998. The utility of cathepsin K inhibitors in the treatment or prevention of resorption in subarticular bone and of generalized bone loss represent a rational approach for pharmacological intervention on the progression of rheumatoid arthritis.

Another embodiment of the invention is a method of treating or preventing the progression of osteoarthritis in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of any of the compounds or any of the pharmaceutical compositions described above. It is known in the literature that osteoarthritis (OA) is accompanied with a well-defined changes in the joints, including erosion of the articular cartilage surface, peri-articular endochondral ossification/osteophytosis, and subchondral bony sclerosis and cyst formation, see Oettmeier R & Abendroth, K, " Osteoarthritis and bone: osteologic types of osteoarthritis of the hip", *Skeletal Radiol.* 18: 165-74, 1989. Recently, the potential contribution of subchondral bone sclerosis to the initiation and

progression of OA have been suggested. Stiffened subchondral bone as the joint responding to repetitive impulsive loading, is less able to attenuate and distribute forces through the joint, subjecting it to greater mechanical stress across the articular cartilage surface. This in turn accelerates cartilage wear and fibrillate. See, Radin, EL and Rose RM, "Role of subchondral bone in the initiation and progression of cartilage damage." *Clin. Orthop.* 213: 34-40, 1986. Inhibition of excessive subarticular bone resorption by an anti-resorptive agent such as a cathepsin K inhibitor, will lead to inhibition of subchondral bone turnover, thus may have a favorable impact on OA progression. In addition to the above hypothesis, cathepsin K protein expression was recently identified in synovial fibroblasts, macrophage-like cells, and chondrocytes from synovium and articular cartilage specimens derived from OA patients. See, Hou, W-S *et al.*, "Comparison of Cathepsin K and S expression within the Rheumatoid and Osteoarthritic Synovium", *Arthritis Rheumatism* 46: 663-74, 2002; and Dodd RA *et al.*, "Expression of Cathepsin K messenger RNA in giant cells and their precursors in human osteoarthritic synovial tissues". *Arthritis Rheumatism* 42: 1588-93, 1999; and Konttinen *et al.*, "Acidic cysteine endoproteinase cathepsin K in the degeneration of the superficial articular hyaline cartilage in osteoarthritis", *Arthritis Rheumatism* 46: 953-60, 2002. These recent studies thus implicated the role of cathepsin K in the destruction of collagen type II in the articular cartilage associated with the progression of osteoarthritis. The utility of cathepsin K inhibitors in the treatment or prevention of osteoarthritis as described in this invention thus comprise of two different mechanisms, one is on the inhibition of osteoclast-driven subchondral bone turnover, and two is on the direct inhibition of collagen type II degeneration in the synovium and cartilage of patients with OA.

Another embodiment of the invention is a method treating cancer in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of any of the compounds or any of the pharmaceutical compositions described above. It is known in the literature that cathepsin K is expressed in human breast carcinoma. See Littlewood-Evans AJ *et al.*, "The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma." *Cancer Res* 57(23):5386-90, December 1, 1997.

Exemplifying the invention is the use of any of the compounds described above in the preparation of a medicament for the treatment and/or prevention of osteoporosis in a mammal in need thereof. Still further exemplifying the invention is the use of any of the compounds described above in the preparation of a medicament for the treatment and/or prevention of: bone loss, bone resorption, bone fractures, metastatic bone disease and/or disorders related to cathepsin functioning.

The compounds of this invention may be administered to mammals, preferably humans, either alone or, preferably, in combination with pharmaceutically acceptable carriers or diluents, optionally with known adjuvants, such as alum, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

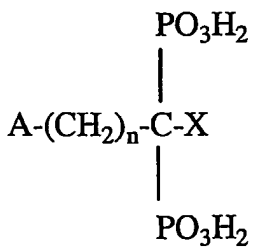
In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch, and lubricating agents, such as magnesium stearate, are commonly added. For oral administration in capsule form, useful diluents include lactose and dried corn starch. For oral use of a therapeutic compound according to this invention, the selected compound may be administered, for example, in the form of tablets or capsules, or as an aqueous solution or suspension. For oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring agents may be added. For intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the active ingredient are usually prepared, and the pH of the solutions should be suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled in order to render the preparation isotonic.

The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Compounds of the invention may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide-phenol, polyhydroxy-ethylaspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polyactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and crosslinked or amphipathic block copolymers of hydrogels.

The instant compounds are also useful in combination with known agents useful for treating or preventing osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures, rheumatoid arthritis, osteoarthritis, periprosthetic osteolysis, osteogenesis imperfecta, obesity, atherosclerosis, chronic obstructive pulmonary disease, juvenile onset diabetes, multiple sclerosis, pemphigus vulgaris, Graves' disease, myasthenia gravis, systemic lupus erythemotasus, rheumatoid arthritis and Hashimoto's thyroiditis, asthma, allogenic immune responses, parasitic infection, cancer, metastatic bone disease, hypercalcemia of malignancy or multiple myeloma. Combinations of the presently disclosed compounds with other agents useful in treating or preventing osteoporosis or other bone disorders are within the scope of the invention. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the disease involved. Such agents include the following: an organic bisphosphonate; an estrogen receptor modulator; an androgen receptor modulator; an inhibitor of osteoclast proton ATPase; an inhibitor of HMG-CoA reductase; an integrin receptor antagonist; an osteoblast anabolic agent, such as PTH; and the pharmaceutically acceptable salts and mixtures thereof. A preferred combination is a compound of the present invention and an organic bisphosphonate. Another preferred combination is a compound of the present invention and an estrogen receptor modulator. Another preferred combination is a compound of the present invention and an androgen receptor modulator. Another preferred combination is a compound of the present invention and an osteoblast anabolic agent.

"Organic bisphosphonate" includes, but is not limited to, compounds of the chemical formula



wherein n is an integer from 0 to 7 and wherein A and X are independently selected from the group consisting of H, OH, halogen, NH_2, SH, phenyl, C1-C30 alkyl, C3-C30 branched or cycloalkyl, bicyclic ring structure containing two or three N, C1-C30 substituted alkyl, C1-C10 alkyl substituted NH_2, C3-C10 branched or cycloalkyl substituted NH_2, C1-C10 dialkyl substituted NH_2, C1-C10 alkoxy, C1-C10 alkyl substituted thio, thiophenyl, halophenylthio, C1-C10 alkyl substituted phenyl, pyridyl, furanyl, pyrrolidinyl, imidazolyl, imidazopyridinyl, and benzyl, such that both A and X are not selected from H or

OH when n is 0; or A and X are taken together with the carbon atom or atoms to which they are attached to form a C3-C10 ring.

In the foregoing chemical formula, the alkyl groups can be straight, branched, or cyclic, provided sufficient atoms are selected for the chemical formula. The C1-C30 substituted alkyl can include a wide variety of substituents, nonlimiting examples which include those selected from the group consisting of phenyl, pyridyl, furanyl, pyrrolidinyl, imidazonyl, NH_2, C1-C10 alkyl or dialkyl substituted NH_2, OH, SH, and C1-C10 alkoxy.

The foregoing chemical formula is also intended to encompass complex carbocyclic, aromatic and hetero atom structures for the A and/or X substituents, nonlimiting examples of which include naphthyl, quinolyl, isoquinolyl, adamantyl, and chlorophenylthio.

Pharmaceutically acceptable salts and derivatives of the bisphosphonates are also useful herein. Non-limiting examples of salts include those selected from the group consisting alkali metal, alkaline metal, ammonium, and mono-, di-, tri-, or tetra-C1-C30-alkyl-substituted ammonium. Preferred salts are those selected from the group consisting of sodium, potassium, calcium, magnesium, and ammonium salts. More preferred are sodium salts. Non-limiting examples of derivatives include those selected from the group consisting of esters, hydrates, and amides.

It should be noted that the terms "bisphosphonate" and "bisphosphonates", as used herein in referring to the therapeutic agents of the present invention are meant to also encompass diphosphonates, biphosphonic acids, and diphosphonic acids, as well as salts and derivatives of these materials. The use of a specific nomenclature in referring to the bisphosphonate or bisphosphonates is not meant to limit the scope of the present invention, unless specifically indicated. Because of the mixed nomenclature currently in use by those of ordinary skill in the art, reference to a specific weight or percentage of a bisphosphonate compound in the present invention is on an acid active weight basis, unless indicated otherwise herein. For example, the phrase "about 5 mg of a bone resorption inhibiting bisphosphonate selected from the group consisting of alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof, on an alendronic acid active weight basis" means that the amount of the bisphosphonate compound selected is calculated based on 5 mg of alendronic acid.

Non-limiting examples of bisphosphonates useful herein include the following:
Alendronate, also known as alendronic acid, alendronate sodium, alendronate monosodium trihydrate or 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid monosodium trihydrate.
Alendronate is described in U.S. Patents 4,922,007, to Kieczykowski et al., issued May 1, 1990; 5,019,651, to Kieczykowski et al., issued May 28, 1991; 5,510,517, to Dauer et al., issued April 23, 1996; 5,648,491, to Dauer et al., issued July 15, 1997, all of which are incorporated by reference herein in their entirety.

Cycloheptylaminomethylene-1,1-bisphosphonic acid, YM 175, Yamanouchi (incadronate, formerly known as cimadronate), as described in U.S. Patent 4,970,335, to Isomura et al., issued November 13, 1990, which is incorporated by reference herein in its entirety.

1,1-dichloromethylene-1,1-diphosphonic acid (clodronic acid), and the disodium salt (clodronate, Procter and Gamble), are described in Belgium Patent 672,205 (1966) and *J. Org. Chem 32*, 4111 (1967), both of which are incorporated by reference herein in their entirety.

1-hydroxy-3-(1-pyrrolidinyl)-propylidene-1,1-bisphosphonic acid (EB-1053).

1-hydroxyethane-1,1-diphosphonic acid (etidronic acid).

1-hydroxy-3-(N-methyl-N-pentylamino)propylidene-1,1-bisphosphonic acid, also known as BM-210955, Boehringer-Mannheim (ibandronate), is described in U.S. Patent No. 4,927,814, issued May 22, 1990, which is incorporated by reference herein in its entirety.

1-hydroxy-2-imidazo-(1,2-a)pyridin-3-yethylidene (minodronate).

6-amino-1-hydroxyhexylidene-1,1-bisphosphonic acid (neridronate).

3-(dimethylamino)-1-hydroxypropylidene-1,1-bisphosphonic acid (olpadronate).

3-amino-1-hydroxypropylidene-1,1-bisphosphonic acid (pamidronate).

[2-(2-pyridinyl)ethylidene]-1,1-bisphosphonic acid (piridronate) is described in U.S. Patent No. 4,761,406, which is incorporated by reference in its entirety.

1-hydroxy-2-(3-pyridinyl)-ethylidene-1,1-bisphosphonic acid (risedronate).

(4-chlorophenyl)thiomethane-1,1-disphosphonic acid (tiludronate) as described in U.S. Patent 4,876,248, which is incorporated by reference herein in its entirety.

1-hydroxy-2-(1H-imidazol-1-yl)ethylidene-1,1-bisphosphonic acid (zoledronate).

Nonlimiting examples of bisphosphonates include alendronate, cimadronate, clodronate, etidronate, ibandronate, incadronate, minodronate, neridronate, olpadronate, pamidronate, piridronate, risedronate, tiludronate, and zolendronate, and pharmaceutically acceptable salts and esters thereof. A particularly preferred bisphosphonate is alendronate, especially a sodium, potassium, calcium, magnesium or ammonium salt of alendronic acid. Exemplifying the preferred bisphosphonate is a sodium salt of alendronic acid, especially a hydrated sodium salt of alendronic acid. The salt can be hydrated with a whole number of moles of water or non whole numbers of moles of water. Further exemplifying the preferred bisphosphonate is a hydrated sodium salt of alendronic acid, especially when the hydrated salt is alendronate monosodium trihydrate.

It is recognized that mixtures of two or more of the bisphosphonate actives can be utilized.

The precise dosage of the organic bisphosphonate will vary with the dosing schedule, the particular bisphosphonate chosen, the age, size, sex and condition of the mammal or human, the nature and severity of the disorder to be treated, and other relevant medical and physical factors. Thus, a precise pharmaceutically effective amount cannot be specified in advance and can be readily determined by the

caregiver or clinician. Appropriate amounts can be determined by routine experimentation from animal models and human clinical studies. Generally, an appropriate amount of bisphosphonate is chosen to obtain a bone resorption inhibiting effect, *i.e.* a bone resorption inhibiting amount of the bisphosphonate is administered. For humans, an effective oral dose of bisphosphonate is typically from about 1.5 to about 6000 μg/kg body weight and preferably about 10 to about 2000 μg/kg of body weight. For alendronate monosodium trihydrate, common human doses which are administered are generally in the range of about 2 mg/day to about 40 mg/day, preferably about 5 mg/day to about 40 mg/day. In the U.S. presently approved dosages for alendronate monosodium trihydrate are 5 mg/day for preventing osteoporosis, 10 mg/day for treating osteoporosis, and 40 mg/day for treating Paget's disease.

In alternative dosing regimens, the bisphosphonate can be administered at intervals other than daily, for example once-weekly dosing, twice-weekly dosing, biweekly dosing, and twice-monthly dosing. In a once weekly dosing regimen, alendronate monosodium trihydrate would be administered at dosages of 35 mg/week or 70 mg/week.

"Selective estrogen receptor modulators" refers to compounds which interfere or inhibit the binding of estrogen to the receptor, regardless of mechanism. Examples of estrogen receptor modulators include, but are not limited to, estrogen, progestogen, estradiol, droloxifene, raloxifene, lasofoxifene, TSE-424, tamoxifen, idoxifene, LY353381, LY117081, toremifene, fulvestrant, 4-[7-(2,2-dimethyl-1-oxopropoxy-4-methyl-2-[4-[2-(1-piperidinyl)ethoxy]phenyl]-2H-1-benzopyran-3-yl]-phenyl-2,2-dimethylpropanoate, 4,4'-dihydroxybenzophenone-2,4-dinitrophenyl-hydrazone, and SH646.

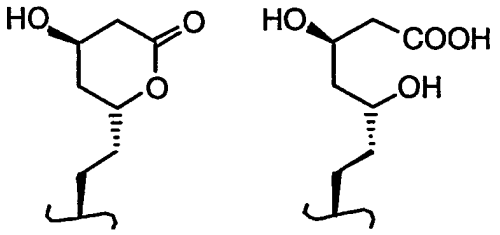
An "estrogen receptor beta modulator" is a compound that selectively agonizes or antagonizes estrogen receptor beta (ER□ Agonizing ER□ increases transcription of the tryptophan hydroxylase gene (TPH, the key enzyme in serotonin synthesis) via an ER□ mediated event. Examples of estrogen receptor beta agonists can be found in PCT International publication WO 01/82923, which published on Novembwer 08, 2001, and WO 02/41835, which published on May 20, 2002, both of which are hereby incorporated by reference in their entirety.

"Androgen receptor modulators" refers to compounds which interfere or inhibit the binding of androgens to the receptor, regardless of mechanism. Examples of androgen receptor modulators include finasteride and other 5α-reductase inhibitors, nilutamide, flutamide, bicalutamide, liarozole, and abiraterone acetate.

"An inhibitor of osteoclast proton ATPase" refers to an inhibitor of the proton ATPase, which is found on the apical membrane of the osteoclast, and has been reported to play a significant role in the bone resorption process. This proton pump represents an attractive target for the design of inhibitors of bone resorption which are potentially useful for the treatment and prevention of osteoporosis and related metabolic diseases. See C. Farina *et al.*, "Selective inhibitors of the osteoclast vacuolar proton ATPase as novel bone antiresorptive agents," *DDT*, 4: 163-172 (1999)), which is hereby incorporated by reference in its entirety.

"HMG-CoA reductase inhibitors" refers to inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase. Compounds which have inhibitory activity for HMG-CoA reductase can be readily identified by using assays well-known in the art. For example, see the assays described or cited in U.S. Patent 4,231,938 at col. 6, and WO 84/02131 at pp. 30-33. The terms "HMG-CoA reductase inhibitor" and "inhibitor of HMG-CoA reductase" have the same meaning when used herein.

Examples of HMG-CoA reductase inhibitors that may be used include but are not limited to lovastatin (MEVACOR®; see U.S. Patent Nos. 4,231,938, 4,294,926 and 4,319,039), simvastatin (ZOCOR®; see U.S. Patent Nos. 4,444,784, 4,820,850 and 4,916,239), pravastatin (PRAVACHOL®; see U.S. Patent Nos. 4,346,227, 4,537,859, 4,410,629, 5,030,447 and 5,180,589), fluvastatin (LESCOL®; see U.S. Patent Nos. 5,354,772, 4,911,165, 4,929,437, 5,189,164, 5,118,853, 5,290,946 and 5,356,896), atorvastatin (LIPITOR®; see U.S. Patent Nos. 5,273,995, 4,681,893, 5,489,691 and 5,342,952) and cerivastatin (also known as rivastatin and BAYCHOL®; see US Patent No. 5,177,080). The structural formulas of these and additional HMG-CoA reductase inhibitors that may be used in the instant methods are described at page 87 of M. Yalpani, "Cholesterol Lowering Drugs", *Chemistry & Industry*, pp. 85-89, February 5, 1996 and US Patent Nos. 4,782,084 and 4,885,314. The term HMG-CoA reductase inhibitor as used herein includes all pharmaceutically acceptable lactone and open-acid forms (i.e., where the lactone ring is opened to form the free acid) as well as salt and ester forms of compounds which have HMG-CoA reductase inhibitory activity, and therefor the use of such salts, esters, open-acid and lactone forms is included within the scope of this invention. An illustration of the lactone portion and its corresponding open-acid form is shown below as structures I and II.



Lactone
I

Open-Acid
II

In HMG-CoA reductase inhibitors where an open-acid form can exist, salt and ester forms may preferably be formed from the open-acid, and all such forms are included within the meaning of the term "HMG-CoA reductase inhibitor" as used herein. Preferably, the HMG-CoA reductase inhibitor is selected from lovastatin and simvastatin, and most preferably simvastatin. Herein, the term "pharmaceutically acceptable salts" with respect to the HMG-CoA reductase inhibitor shall mean non-toxic salts of the compounds employed in this invention which are generally prepared by reacting the free acid with a suitable organic or inorganic base, particularly those formed from cations such as sodium,

potassium, aluminum, calcium, lithium, magnesium, zinc and tetramethylammonium, as well as those salts formed from amines such as ammonia, ethylenediamine, N-methylglucamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, 1-p-chlorobenzyl-2-pyrrolidine-1'-yl-methylbenz-imidazole, diethylamine, piperazine, and tris(hydroxymethyl) aminomethane. Further examples of salt forms of HMG-CoA reductase inhibitors may include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamaote, palmitate, panthothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate.

Ester derivatives of the described HMG-CoA reductase inhibitor compounds may act as prodrugs which, when absorbed into the bloodstream of a warm-blooded animal, may cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy.

As used above, "integrin receptor antagonists" refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha_V\beta_3$ integrin, to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha_V\beta_5$ integrin, to compounds which antagonize, inhibit or counteract binding of a physiological ligand to both the $\alpha_V\beta_3$ integrin and the $\alpha_V\beta_5$ integrin, and to compounds which antagonize, inhibit or counteract the activity of the particular integrin(s) expressed on capillary endothelial cells. The term also refers to antagonists of the $\alpha_V\beta_6$, $\alpha_V\beta_8$, $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$ and $\alpha_6\beta_4$ integrins. The term also refers to antagonists of any combination of $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_V\beta_6$, $\alpha_V\beta_8$, $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$ and $\alpha_6\beta_4$ integrins. H.N. Lode *et al.*, *PNAS USA* 96: 1591-1596, 1999 have observed synergistic effects between an antiangiogenic αv integrin antagonist and a tumor-specific antibody-cytokine (interleukin-2) fusion protein in the eradication of spontaneous tumor metastases. Their results suggested this combination as having potential for the treatment of cancer and metastatic tumor growth. $\alpha_v\beta_3$ integrin receptor antagonists inhibit bone resorption through a new mechanism distinct from that of all currently available drugs. Integrins are heterodimeric transmembrane adhesion receptors that mediate cell-cell and cell-matrix interactions. The α and β integrin subunits interact non-covalently and bind extracellular matrix ligands in a divalent cation-dependent manner. The most abundant integrin on osteoclasts is $\alpha_V\beta_3$ ($>10^7$/osteoclast), which appears to play a rate-limiting role in cytoskeletal organization important for cell migration and polarization. The $\alpha_V\beta_3$ antagonizing effect is selected from inhibition of bone resorption, inhibition of restenosis, inhibition of macular degeneration, inhibition of arthritis, and inhibition of cancer and metastatic growth.

"An osteoblast anabolic agent" refers to agents that build bone, such as PTH. The intermittent administration of parathyroid hormone (PTH) or its amino-terminal fragments and analogues have been shown to prevent, arrest, partially reverse bone loss and stimulate bone formation in animals and humans. For a discussion refer to D.W. Dempster *et al.*, "Anabolic actions of parathyroid hormone on bone," *Endocr Rev* 14: 690-709, 1993. Studies have demonstrated the clinical benefits of parathyroid hormone in stimulating bone formation and thereby increasing bone mass and strength. Results were reported by RM Neer *et al., New Eng J Med* 344 1434-1441, 2001.

In addition, parathyroid hormone-related protein fragments or analogues, such as PTHrP-(1-36) have demonstrated potent anticalciuric effects [see M.A. Syed *et al.*, "Parathyroid hormone-related protein-(1-36) stimulates renal tubular calcium reabsorption in normal human volunteers: implications for the pathogenesis of humoral hypercalcemia of malignancy," *JCEM* 86: 1525-1531 (2001)] and may also have potential as anabolic agents for treating osteoporosis.

If formulated as a fixed dose, such combination products employ the compounds of this invention within the dosage range described below and the other pharmaceutically active agent(s) within its approved dosage range. Compounds of the instant invention may alternatively be used sequentially with known pharmaceutically acceptable agent(s) when a combination formulation is inappropriate.

The term "administration" and variants thereof (e.g., "administering" a compound) in reference to a compound of the invention means introducing the compound or a prodrug of the compound into the system of the animal in need of treatment. When a compound of the invention or prodrug thereof is provided in combination with one or more other active agents (e.g., a cytotoxic agent, etc.), "administration" and its variants are each understood to include concurrent and sequential introduction of the compound or prodrug thereof and other agents. The present invention includes within its scope prodrugs of the compounds of this invention. In general, such prodrugs will be functional derivatives of the compounds of this invention which are readily convertible *in vivo* into the required compound. Thus, in the methods of treatment of the present invention, the term "administering" shall encompass the treatment of the various conditions described with the compound specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound *in vivo* after administration to the patient. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs," ed. H. Bundgaard, Elsevier, 1985, which is incorporated by reference herein in its entirety. Metabolites of these compounds include active species produced upon introduction of compounds of this invention into the biological milieu.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which

results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

The term "therapeutically effective amount" as used herein means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician.

The terms "treating" or "treatment" of a disease as used herein includes: preventing the disease, i.e. causing the clinical symptoms of the disease not to develop in a mammal that may be exposed to or predisposed tothe disease but does not yet experience or display symptoms of the disease; inhibiting the disease, i.e., arresting or reducing the development of the disease or its clinical symptoms; or relieving the disease, i.e., causing regression of the disease or its clinical symptoms.

The term "bone resorption," as used herein, refers to the process by which osteoclasts degrade bone.

The present invention also encompasses a pharmaceutical composition useful in the treatment of osteoporosis or other bone disorders, comprising the administration of a therapeutically effective amount of the compounds of this invention, with or without pharmaceutically acceptable carriers or diluents. Suitable compositions of this invention include aqueous solutions comprising compounds of this invention and pharmacologically acceptable carriers, *e.g.*, saline, at a pH level, *e.g.*, 7.4. The solutions may be introduced into a patient's bloodstream by local bolus injection.

When a compound according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, and response of the individual patient, as well as the severity of the patient's symptoms.

In one exemplary application, a suitable amount of compound is administered to a mammal undergoing treatment for a cathepsin dependent condition. Oral dosages of the present invention, when used for the indicated effects, will range between about 0.01 mg per kg of body weight per day (mg/kg/day) to about 100 mg/kg/day, preferably 0.01 to 10 mg/kg/day, and most preferably 0.1 to 5.0 mg/kg/day. For oral administration, the compositions are preferably provided in the form of tablets containing 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100 and 500 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably, from about 1 mg to about 100 mg of active ingredient. Intravenously, the most preferred doses will range from about 0.1 to about 10 mg/kg/minute during a constant rate infusion. Advantageously, compounds of the present

invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, preferred compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittant throughout the dosage regimen.

The compounds of the present invention can be used in combination with other agents useful for treating cathepsin-mediated conditions. The individual components of such combinations can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly. It will be understood that the scope of combinations of the compounds of this invention with other agents useful for treating cathepsin-mediated conditions includes in principle any combination with any pharmaceutical composition useful for treating disorders related to estrogen functioning.

The scope of the invetion therefore encompasses the use of the instantly claimed compounds in combination with a second agent selected from: an organic bisphosphonate; an estrogen receptor modulator; an androgen receptor modulator; an inhibitor of osteoclast proton ATPase; an inhibitor of HMG-CoA reductase; an integrin receptor antagonist; an osteoblast anabolic agent, such as PTH; and the pharmaceutically acceptable salts and mixtures thereof.

These and other aspects of the invention will be apparent from the teachings contained herein.

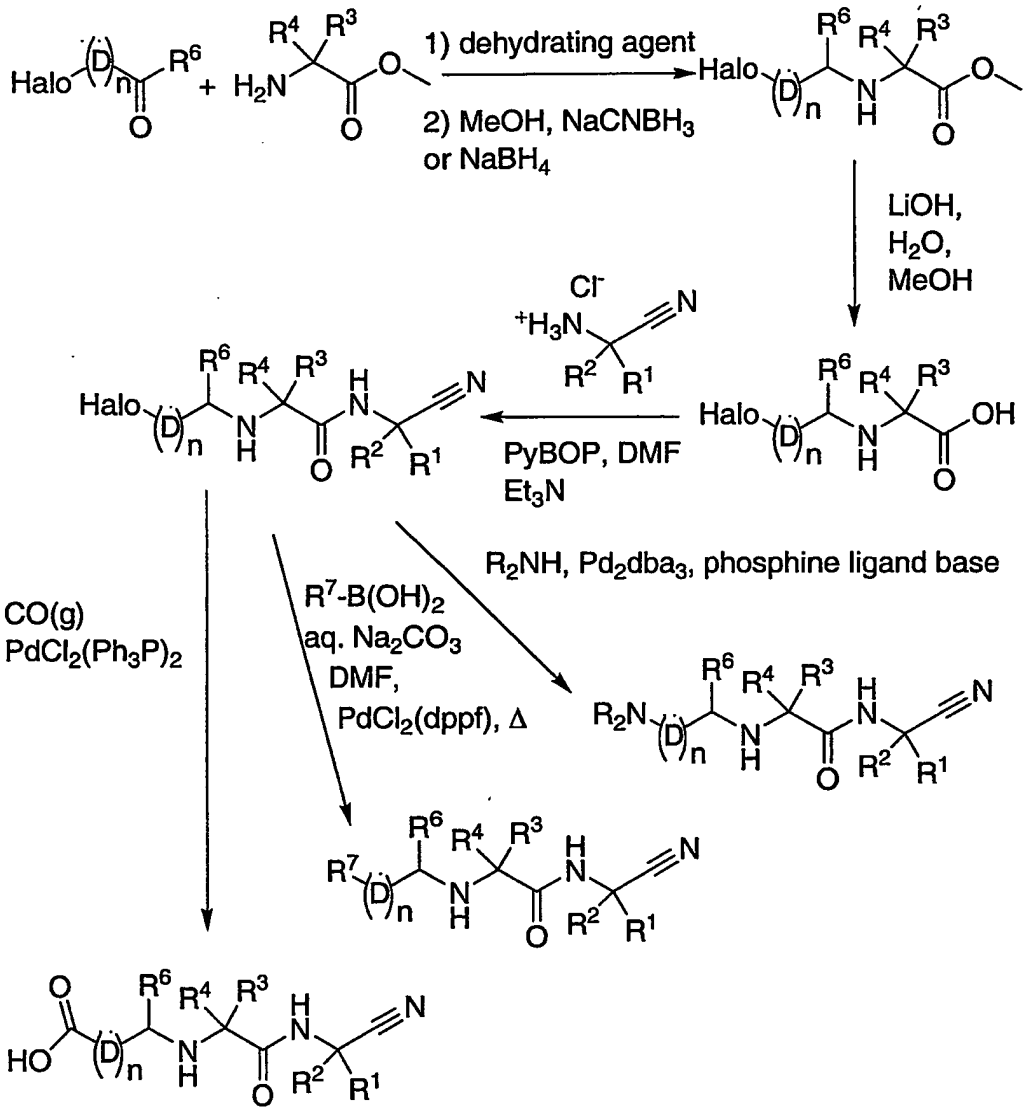
The details of one or more embodiments of the invention are set forth in the accompanying description above. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All patents and publications cited in this specification are incorporated by reference.

The foregoing description has been presented only for the purposes of illustration and is not intended to limit the invention to the precise form disclosed, but by the claims appended hereto.

SCHEMES

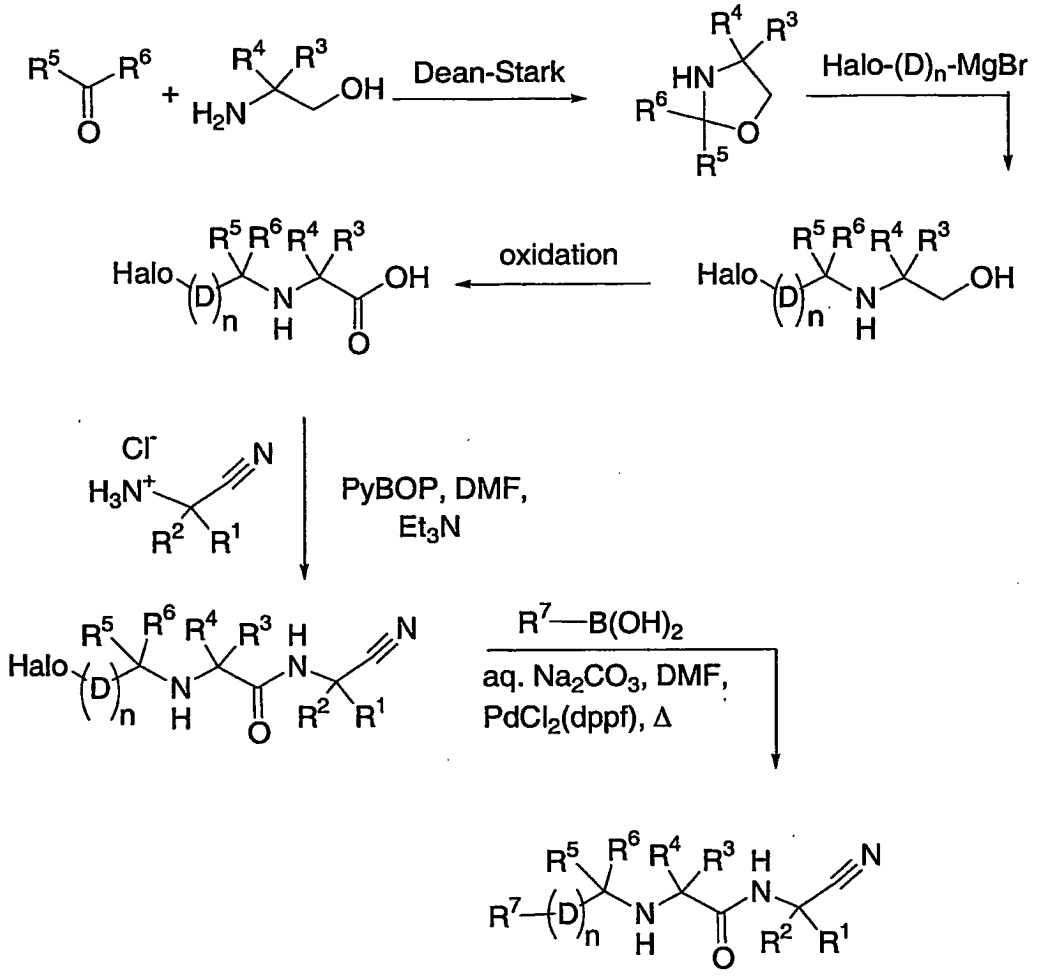
Compounds of the present invention can be prepared according to Scheme 1, as indicated below. Thus an α-amino ester may be added to a haloalkyl ketone to form an aminal which may be dehydrated to an imine in the presence of a dehydrating agent such as $TiCl_4$, $MgSO_4$ or isopropyl trifluoroacetate. Reduction of the imine with a reducing agent such as sodium cyanoborohydride or sodium borohydride provides the amine. Ester hydrolysis and amide formation with an appropriately substituted aminoacetonitrile provides compounds of the current invention. If the substituent on D system is a halogen, a palladium-catalyzed Suzuki coupling with an appropriate boronic acid provides additional compounds of the current invention. Alternatively, a copper-catalyzed or palladium-catalyzed Buchwald coupling with a suitable amine provides additional compounds of the current invention. Alternatively, a palladium-catalyzed caboxylation followed by amide formation with a suitable amine provides additional compounds of the current invention.

SCHEME 1



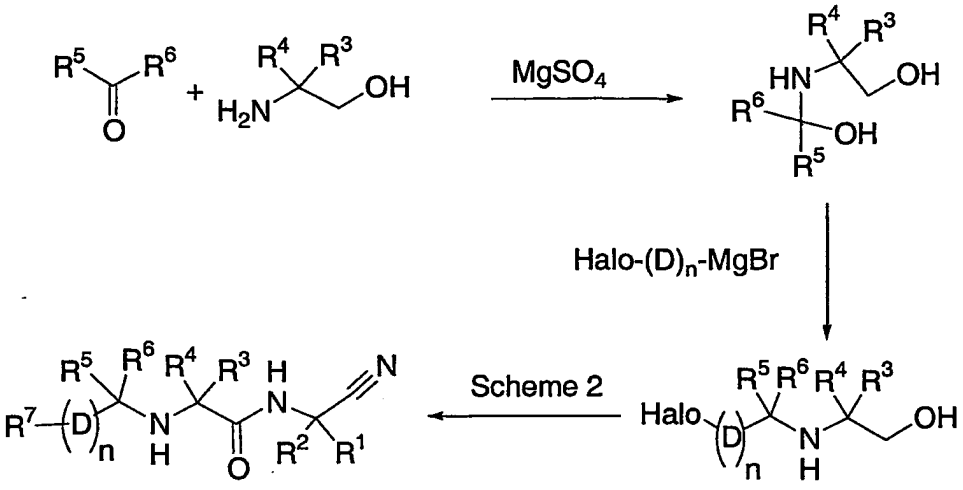
Compounds of the present invention may also be prepared according to Scheme 2, as indicated below. A ketone or aldehyde may be condensed with an amino alcohol to give a cyclic aminal. Treatment with 3 equivalents of a Grignard reagent or organolithium reagent will provide the appropriate alkylated amino alcohol. Oxidation of the alcohol with a chromium system such as a Jones oxidation or H_5IO_6/CrO_3, or alternatively by a two-step oxidation (eg oxalyl chloride/ DMSO/Et_3N followed by NaClO) will provide the corresponding carboxylic acid. Peptide coupling and Suzuki reaction as described in Scheme 1 will provide compounds of the current invention.

SCHEME 2



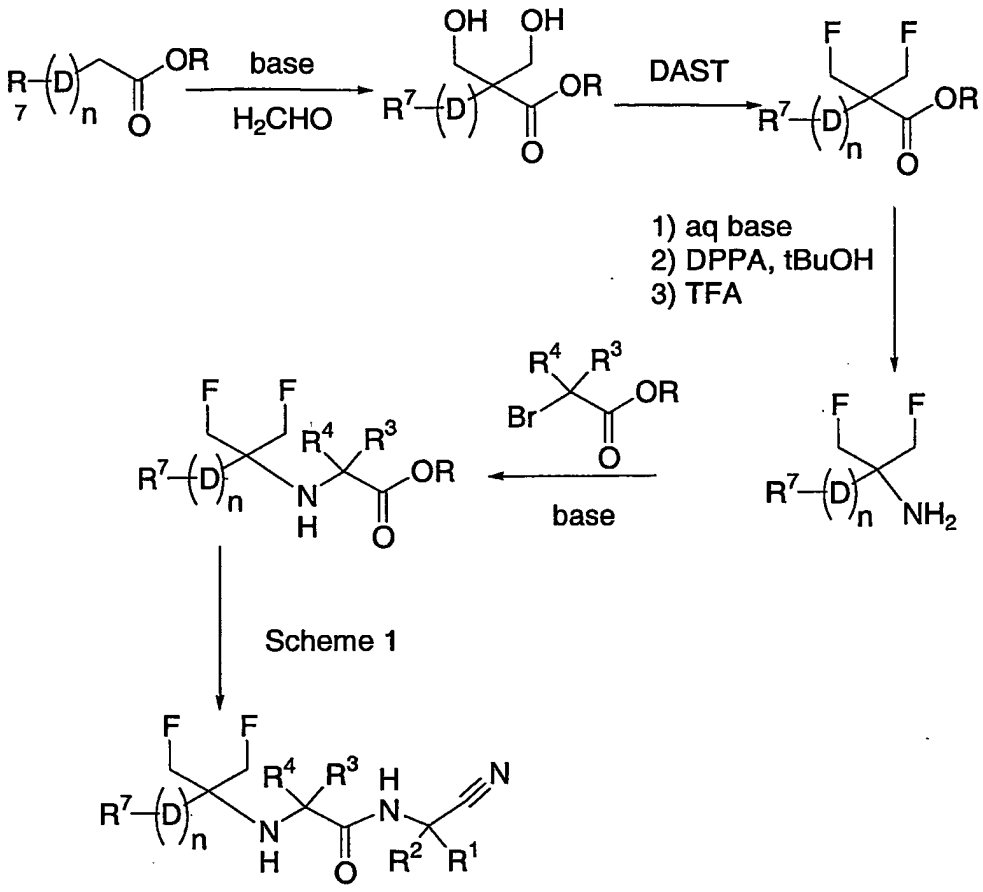
Compounds of the present invention may also be prepared according to Scheme 3, as indicated below. A ketone or aldehyde may be condensed with an amino alcohol to give an acyclic aminal. Treatment with multiple equivalents of a Grignard reagent or organolithium reagent will provide the appropriate alkylated amino alcohol. This alcohol can be converted into compounds of the current invention by the method described in Scheme 2.

SCHEME 3



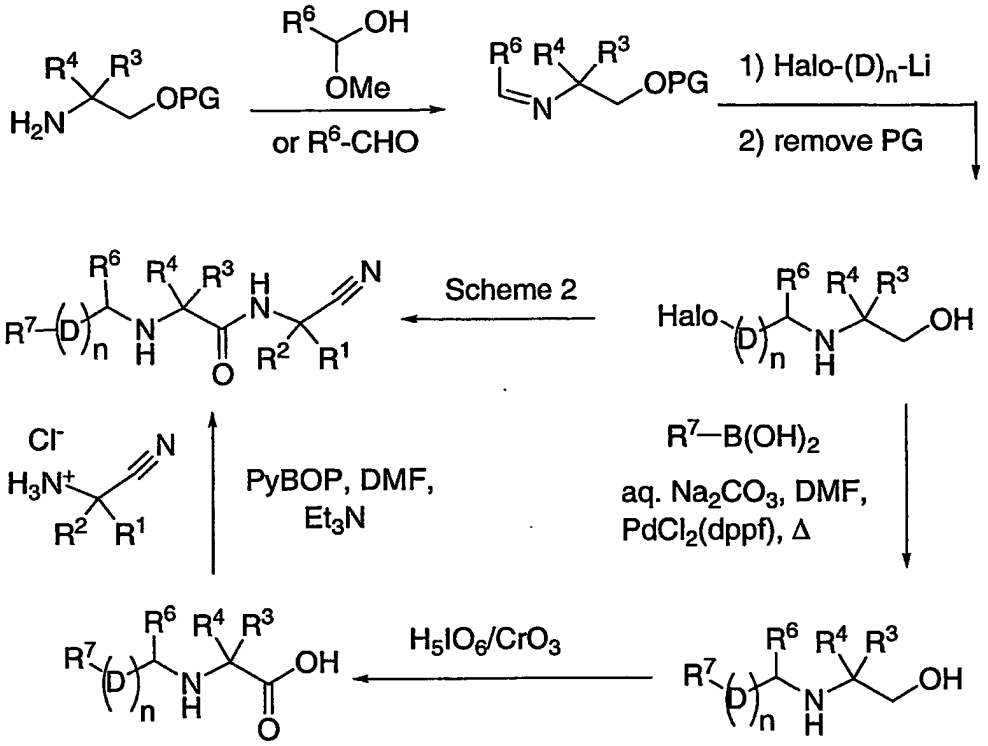
Compounds of the current invention may also be prepared according to Scheme 4. An appropriately substituted acetate may be enolized with a suitable base (including, but not limited to LDA, KHMDS, NaH or nBuLi) and treated with paraformaldehyde to generate the diol. This diol may be converted to the difluoride using a fluorinating reagent such as DAST. Hydrolysis of the ester followed by Curtius rearrangement will then provide the amine. This amine can displace an appropriately substituted alpha-bromo ester to provide the alpha-amino ester. This may be converted into compounds of the current invention by the method described in Scheme 1.

SCHEME 4



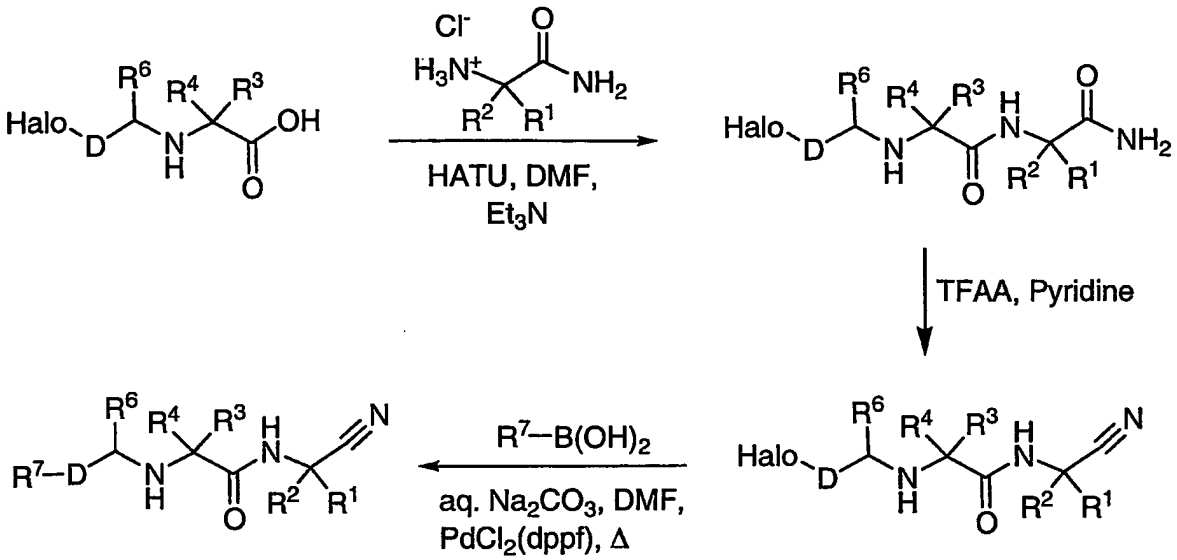
Compounds of the current invention may also be prepared according to Scheme 5, as indicated below. An aldehyde or a hemiacetal may be condensed with an amino alcohol with azeotropic removal of water in which the alcohol moiety is protected with a suitable protecting group. Treatment of the resulting imine with a Grignard reagent or organolithium reagent will provide the appropriate alkylated amino alcohol. The alcohol protecting group can then be removed and the alcohol can be converted into compounds of the current invention either by the method described in Scheme 2 or by first conducting the Suzuki reaction, followed by oxidizing the alcohol with H_5IO_6 /CrO_3 and then peptide coupling.

SCHEME 5



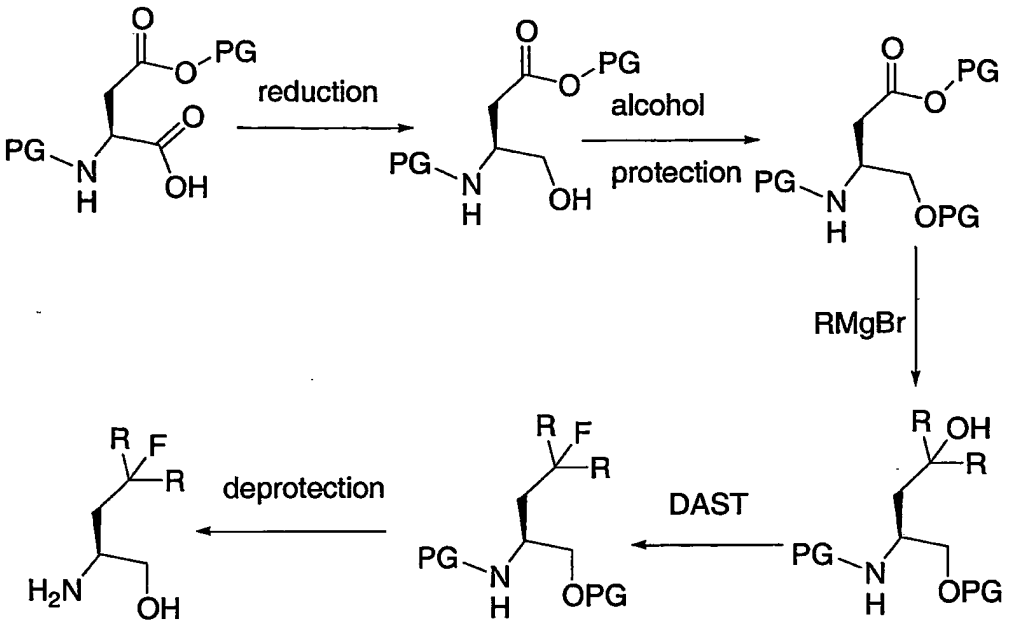
Compounds of the current invention may also be prepared according to Scheme 6, as indicated below. The peptide coupling of an alpha-amino acid described in Schemes 1, 2, or 5, with an alpha-amino amide followed by dehydration of the resulting primary amide (Voegel, J. J.; Benner, S. A. *Helv. Chem. Acta* **1996**, *79*, 1863) will provide compounds of the current invention.

SCHEME 6



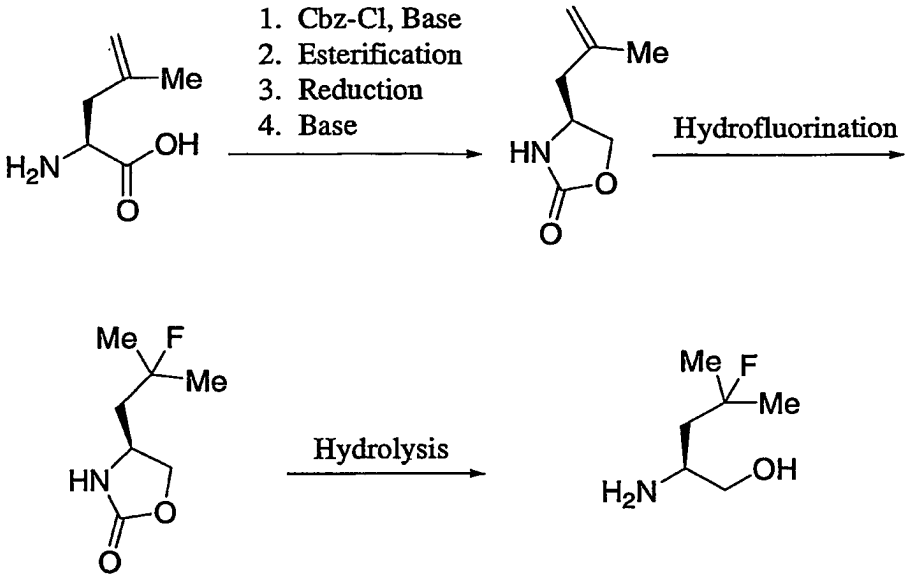
The synthesis of some of the amino alcohols used at the beginning of Schemes 2, 3 and 5 are described in Schemes 7-11. For example, the synthesis of (2*S*)-2-amino-4-fluoro-4-methylpentan-1-ol where R=Me is described in Scheme 7 below. Starting with a suitable diprotected aspartic acid, the carboxy group can be reduced to an alcohol using standard literature procedures (i.e. mixed anyhdride formation followed by $NaBH_4$ reduction). A protected version of 2-amino-4-methylpentane-1,4-diol (R=Me) can then be generated by an appropriate Grignard or organolithiation reaction. Finally, the hydroxy moiety can be converted to the desired fluoro using a fluorinating agent such as DAST. The protected or unprotected version of this amino alcohol can then be converted to compounds of the current invention according to Schemes 1, 2, 3 and 5.

SCHEME 7



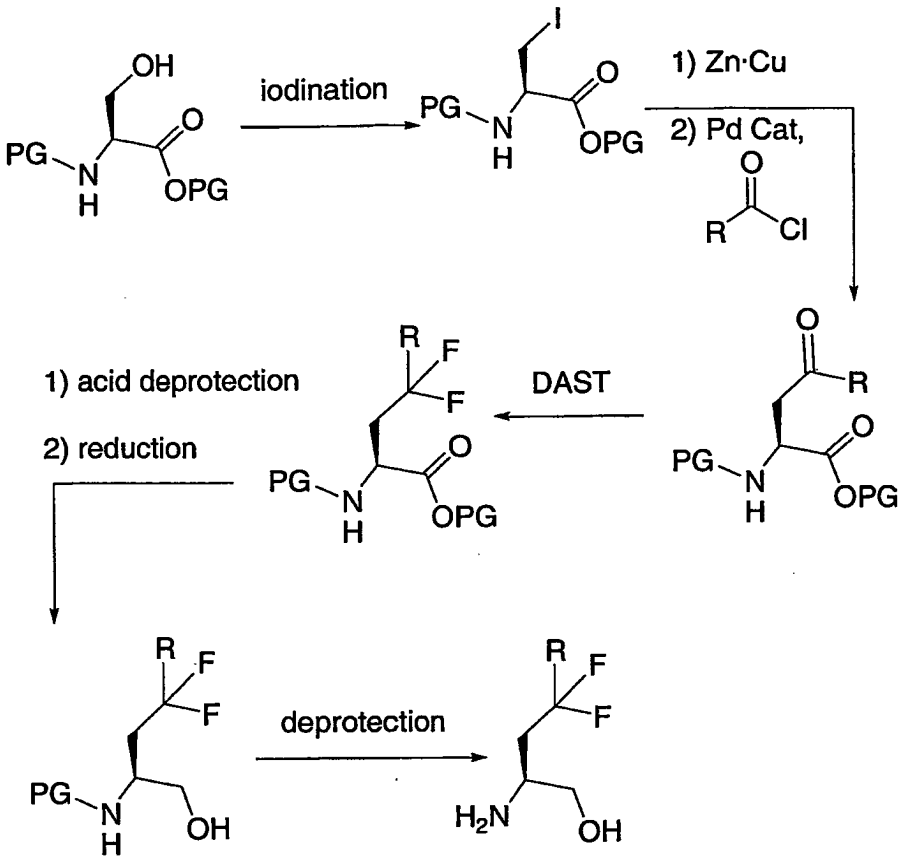
The 4-fluoroleucinol can also be synthesized according to Scheme 8. 4,5-Dehydroleucine is converted to (4*S*)-4-(2-methylprop-2-enyl)-1,3-oxazolidin-2-one as described in the scheme below. This intermediate is then treated with a hydrofluorination reagent such as HF-pyridine to give (4*S*)-4-(2-fluoro-2-methylpropyl)-1,3-oxazolidin-2-one. Basic hydrolysis (i.e. $Ba(OH)_2$ or NaOH) then affords (2*S*)-2-amino-4-fluoro-4-methylpentan-1-ol.

SCHEME 8



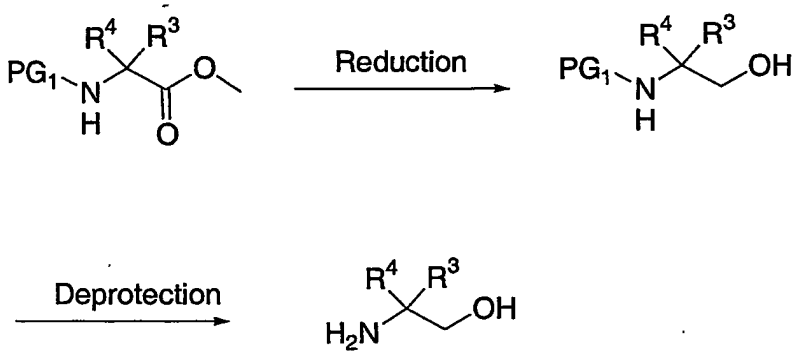
The synthesis of 4,4-difluoro-L-norvaline where R=Me is described in Scheme 9 below. Starting with a suitable diprotected serine, iodination can be carried out using a reagent such as $(PhO)_3P^+MeI^-$. Zincation of the resultant iodide may proceed using Zn·Cu couple and TMSCl. The resultant zincate can then undergo a palladium catalyzed coupling reaction with alkanoyl chloride to generate the ketone. Finally, the ketone moiety can be converted to the desired difluoro derivative using a fluorinating agent such as DAST. The protected or unprotected version of this amino acid or amino alcohol can then be converted to compounds of the current invention according to Schemes 1, 2, 3 and 5.

SCHEME 9



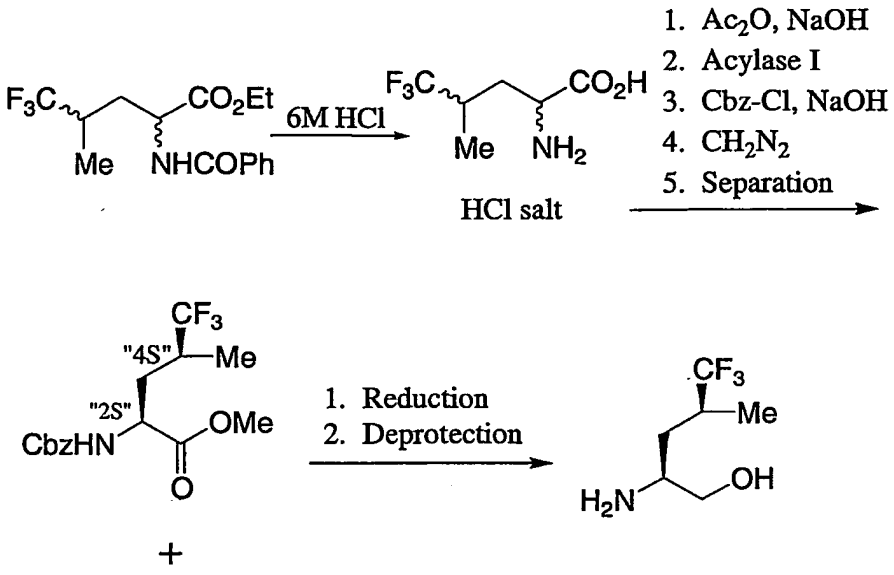
The amino alcohols used for the present invention can also be synthesized according to Scheme 10. A protected amino acid is reduced with a reducing agent such as $NaBH_4$ with or without an additive such as LiCl, in a solvent such as EtOH or a mixed solvent system such as EtOH and THF. The amino protecting group is then removed with the appropriate method according to the nature of the protecting group.

SCHEME 10



Synthesis of (2S,4S)-2-amino-5,5,5-trifluoro-4-methylpentan-1-ol used in the present invention is described in Scheme 11. The N-benzoyl-5,5,5-trifluoroleucine (Ojiima, et. al. J. Org. Chem., **1989**, *54*, 4511 – 4522) can be hydrolysed with an aqueous acid such as 6M HCl under refluxing conditions. The amino acid HCl salt intermediate is then converted to the N-acetyl-5,5,5-trifluoroleucine and the amino group chiral centre is resolved by an enzymatic method (Synthetic Communications, **1996**, *26*, 1109 – 1115.). The isolated 5,5,5-trifluoro-L-leucine is then protected with a protecting group such as benzyl carbamate and the carboxylic acid group is esterified. The two diastereomers at the 4-position are then separated by flash column chromatography. One of the enantiomers, the (2S,4S) protected amino acid is then converted to the amino alcohol as described in scheme 10.

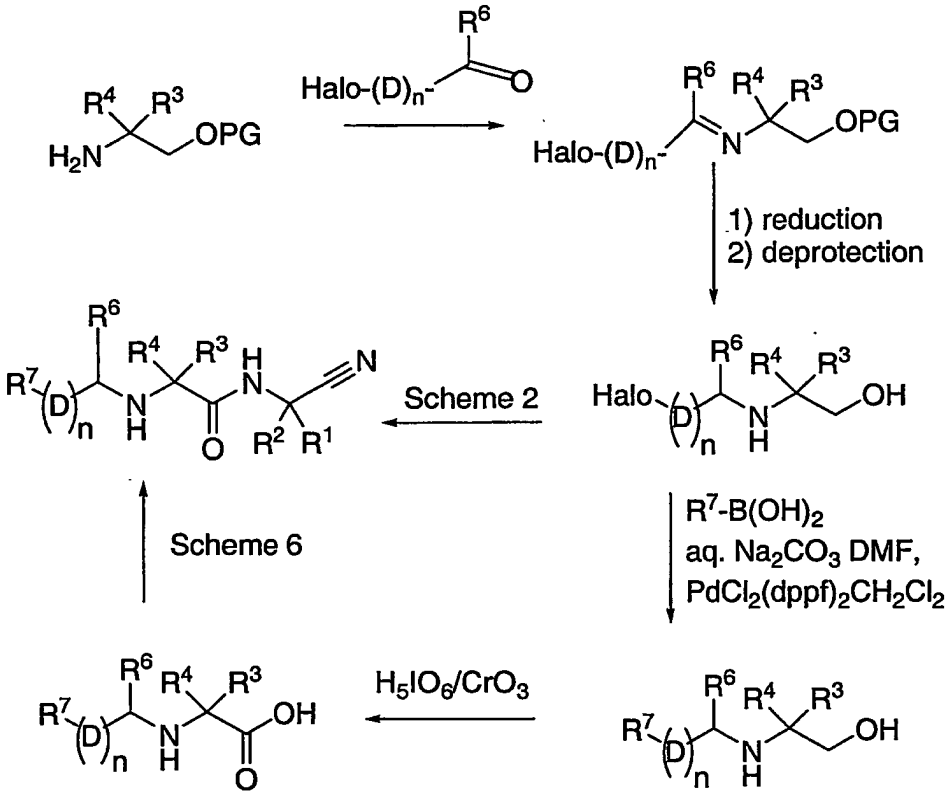
SCHEME 11



2S, 4R- enantiomer

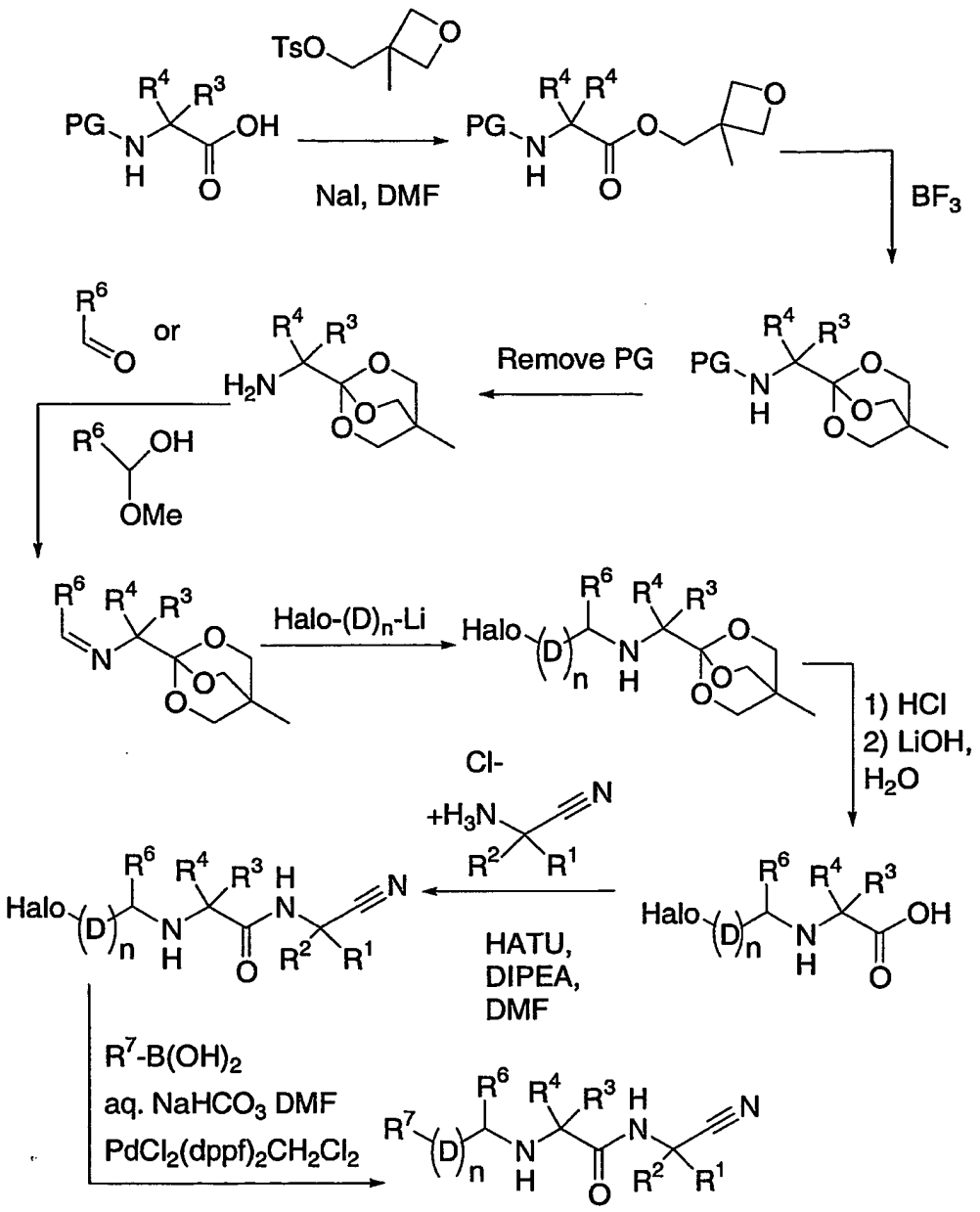
Compounds of the current invention where R^5 is hydrogen and R^6 is aryl or heteroaryl may also be prepared according to Scheme 12 as shown below. Condensation of an aryl or heteroaryl aldehyde with an amino alcohol in which the alcohol moiety is protected with a suitable protecting group, followed by treatment of the resulting imine with a Grignard or organolithium reagent of formula halo-$(D)_n$-Li or halo-$(D)_n$-MgX (where D is as defined in the Summary of the Invention), followed by removal of the oxygen protecting group provides the alkylated aminoalcohol. The alkylated aminoalcohol is then converted into compounds of the current invention either by the method described in Scheme 2 or by first conducting the Suzuki reaction with the boronic ester of the formula R^7-$B(OH)_2$, then oxidizing the alcohol with a suitable oxidizing agent such as H_5IO_6/CrO_3 to give the acid and finally treating the acid with an aminoacetonitrile under peptide coupling conditions as described previously.

SCHEME 12



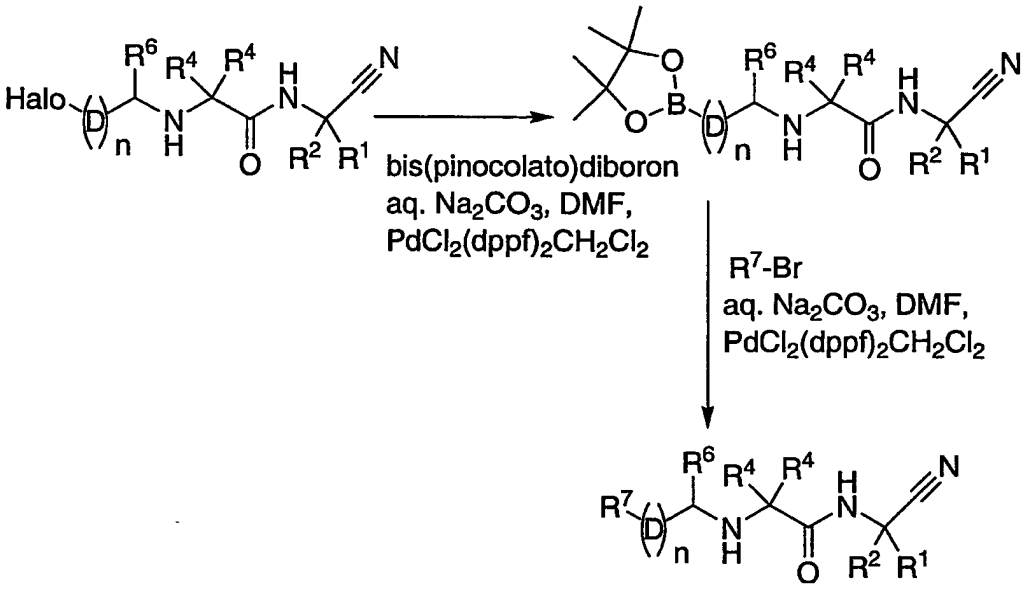
Compounds of the current invention may also be prepared according to Scheme 13, as shown below. Reaction of a suitably N-protected amino acid derivative with oxetane tosylate in the presence of sodium iodide in a suitable organic solvent such as dimethylformamide provides the corresponding oxetane ester which upon treatment with diborane provides the ortho ester. Removal of the amino protecting group affords an amine which upon condensation with an aldehyde of formula R^6CHO (where R^6 is aryl or heteroaryl) or a hemiacetal of formula $R^6C(OH)(OR)$ (where R is an alkyl group) under the reaction conditions described above provides an imine. Treatment of the imine with a Grignard or organolithium reagent under the reaction conditions described above provides an N-alkylated derivative. Removal of the ortho ester provides the corresponding carboxylic acid which is then converted into compounds of the current invention by condensation with an aminoacetonitrile under peptide coupling conditions, followed by Suzuki reaction as described above.

SCHEME 13



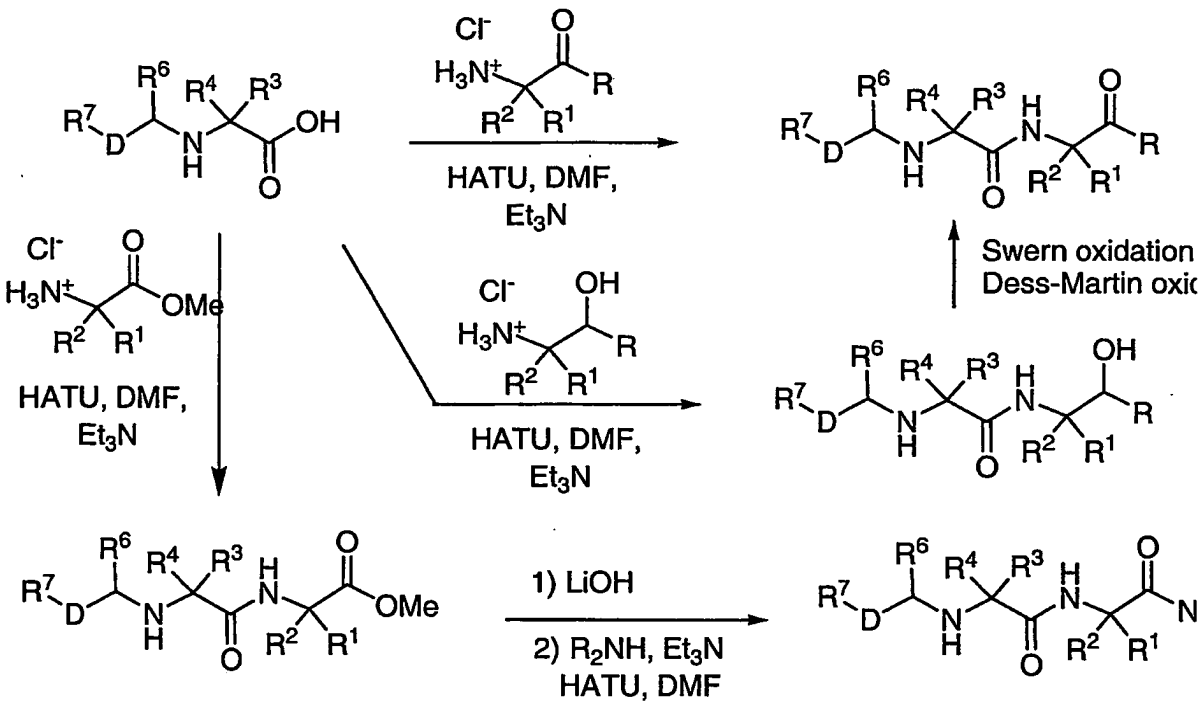
Compounds of the current invention may also be prepared as shown in Scheme 14. A aryl halide containing appropriate R^1, R^2, R^3, R^4 and R^6 groups may be coupled with bis(pinacolato)diboron to give the aryl pinacolate. This may be coupled with R^7-bromides under Suzuki conditions to provide compounds of the current invention.

SCHEME 14



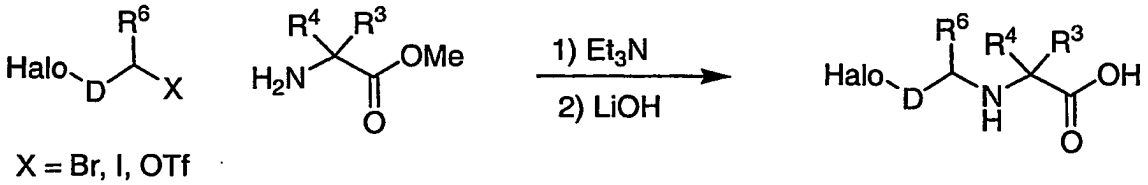
Compounds of the current invention may also be prepared according to Scheme 15, as indicated below. The peptide coupling of an appropriately substituted amino acid described in Schemes 1, 2, or 5, with an alpha-amino ester, alcohol or ketone will provide compounds of the current invention. In the case of an alpha-amino alcohol, oxidation of the product alcohol will provide a ketone which is a compound of the current invention. In the case of an alpha-amino ester, hydrolysis of the product ester followed by amide formation with a suitable amine will provide amides which are compounds of the current invention.

SCHEME 15



Acids shown in Schemes 1, 2, 6 and 15 may also be prepared as shown in Scheme 16. An appropriately substituted benzyl bromide, iodide or triflate (which may be chiral or racemic) may be coupled with an alpha amino ester under basic conditions. Hydrolysis with aqueous base then provides the acid which can be converted into examples of the current invention.

SCHEME 16

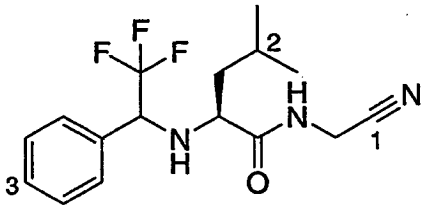


X = Br, I, OTf

The following examples describe the synthesis of selected compounds of the present invention. These examples are illustrative of the invention claimed herein, and are not to be interpretated as limiting the scope of the invention. Table 1 describes how specific examples, when bound in the active site of a cathepsin for which it is active (e.g. Cathepsin K), meet the distance criteria as described herein.

EXAMPLE 1

Synthesis of N^1-(cyanomethyl)-N^2-(2,2,2-trifluoro-1-phenylethyl)-L-leucinamide



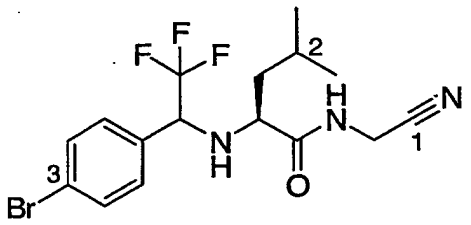
To a solution of L-leucine methyl ester hydrochloride (975 mg, 5.37 mmol) in dichloromethane (30 mL) was added 2,2,2-trifluoroacetophenone (0.75 mL, 5.34 mmol) and diisopropylethylamine (3.5 mL, 20 mmol). $TiCl_4$ (0.55 mL, 5.0 mmol) in 0.45 mL dichloromethane was added dropwise, and the mixture was stirred overnight. Additional $TiCl_4$ (0.4 mL, 3.6 mmol) was then added and the mixture was stirred 3h. A solution of $NaCNBH_3$ (1050 mg, 16.7 mmol) in MeOH (20 mL) was added and the mixture was stirred 2h. Poured into 1N NaOH and extracted with ethyl acetate (2x). The organic phase was washed with 1N NaOH and brine, then dried over $MgSO_4$ and evaporated. Purification by ISCO column chromatography (gradient 30% to 90% ethyl acetate/hexanes) provided methyl *N*-(2,2,2-trifluoro-1-phenylethyl)-L-leucinate.

To a room temperature solution of methyl *N*-(2,2,2-trifluoro-1-phenylethyl)-L-leucinate (150 mg, 0.50 mmol) in 2:1 THF/MeOH was added 1M LiOH. The mixture was stirred overnight and concentrated. The residue was partitioned between ethyl acetate and pH 3.5 phosphate buffer. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated to give *N*-(2,2,2-trifluoro-1-phenylethyl)-L-leucine.

A mixture of *N*-(2,2,2-trifluoro-1-phenylethyl)-L-leucine (149 mg, 0.50 mmol), aminoacetonitrile hydrochloride (102 mg, 1.1 mmol) and PyBOP (260 mg, 0.50 mmol) was dissolved in DMF (5 mL). Triethylamine (0.3 mL, 2.1 mmol) was added and the mixture was stirred overnight, then poured into pH 3 phosphate buffer and extracted with 3:1 ether/ethyl acetate. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated. Purification by ISCO column chromatography (gradient 20% to 50% ethyl acetate/hexanes) provided N^1-(cyanomethyl)-N^2-(2,2,2-trifluoro-1-phenylethyl)-L-leucinamide as a 1:1 mixture of diastereomers.
MS (+APCI): 313.9 [M+1].

EXAMPLE 2

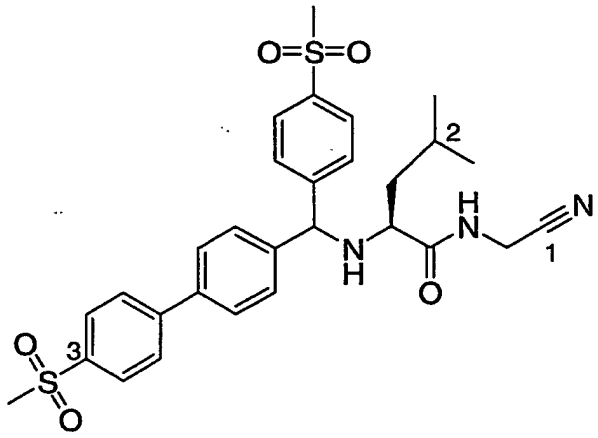
Synthesis of N^2-[1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(cyanomethyl)-L-leucinamide



Using the method of Example 1, N^2-[1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(cyanomethyl)-L-leucinamide was prepared.
MS (-ESI): 403.9, 405.9 $[M-1]^-$

EXAMPLE 3

Synthesis of *N*^{1}-(Cyanomethyl)-*N*^{2}-{[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl][4-(methylsulfonyl)phenyl]methyl}-L-Leucinamide



Step 1: Methyl *N*-{(4-bromophenyl)[4-(methylsulfonyl)phenyl]methylene}-L-leucinate

A solution of (4-bromophenyl)[4-(methylsulfonyl)phenyl]methanone (202 mg, 0.59 mmol), L-leucine methyl ester hydrochloride (328 mg, 2.0 mmol) and camphor sulfonic acid (52 mg, 0.22 mmol) in toluene was refluxed for 18 hours using a Dean-Stark trap. The solvent was removed *in vacuo* and the resulting residue was purified by chromatography using EtOAc and hexane as eluant to

give a 1:1 mixture of the title compound and the starting (4-bromophenyl)[4-(methylsulfonyl)phenyl]methanone.

Step 2: Methyl *N*-{(4-bromophenyl)[4-(methylsulfonyl)phenyl]methyl}-L-leucinate

To a solution of a 1:1 mixture of methyl *N*-{(4-bromophenyl)[4-(methylsulfonyl)phenyl]methylene}leucinate and (4-bromophenyl)[4-(methylsulfonyl)phenyl]methanone from step 1 (185 mg, ~0.2 mmol) in acetic acid/methanol (1:3, 4 mL) was added sodium borohydride (~400 mg) by portions every 30 min over 2 days (addition was stopped during the night) using a solid addition funnel. The reaction mixture was partitioned between EtOAc and water, the organic layer was dried over Na_2SO_4 and concentrated. The resulting mixture was purified by chromatography using EtOAc and hexane as eluant. Methyl *N*-{(4-bromophenyl)[4-(methylsulfonyl)phenyl]methyl}-L-leucinate was obtained as a colorless gum and (4-bromophenyl)[4-(methylsulfonyl)phenyl]methanol was obtained as a white solid.

Step 3: *N*-{(4-bromophenyl)[4-(methylsulfonyl)phenyl]methyl}-L-leucine

To a solution of methyl *N*-{(4-bromophenyl)[4-(methylsulfonyl)phenyl]methyl}-L-leucinate from step 2 (81 mg, 0.17 mmol) in THF (1 mL) and MeOH (0.5 mL) was added 1N LiOH (0.3 mL, 0.3 mmol). The resulting mixture was stirred at room temperature for 18 hours and then partitioned between EtOAc and water + 1N HCl (0.5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give the title compound as a colorless gum.

Step 4: N^2-{(4-bromophenyl)[4-(methylsulfonyl)phenyl]methyl}-N^1-(cyanomethyl)-L-leucinamide

To a solution of *N*-{(4-bromophenyl)[4-(methylsulfonyl)phenyl]methyl}-L-leucine from step 3 (76 mg, 0.17 mmol), HATU (146 mg, 0.38 mmol), aminoacetonitrile hydrochloride (52 mg, 0.56 mmol) in DMF (1.1 mL) cooled to –10 °C, was added *N,N*-diisopropylethylamine (0.13 mL, 0.75 mmol). The reaction was allowed to proceed at room temperature for 18 h and it was partitioned between EtOAc and water. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by chromatography using EtOAc and hexane as eluant to give the title compound as a colorless gum.

Step 5: N^1-(cyanomethyl)-N^2-{[4-(methylsulfonyl)phenyl][4'-(methylthio)-1,1'-biphenyl-4-yl]methyl}-L-leucinamide

A heterogeneous mixture of N^2-{(4-bromophenyl)[4-(methylsulfonyl)phenyl]methyl}-N^1-(cyanomethyl)-L-leucinamide from step 4 (72 mg, 0.15 mmol), 4-(methylthio)phenylboronic acid (37

mg, 0.22 mmol) in ethylene glycol dimethyl ether (1mL) and 2M aqueous sodium carbonate was degassed under vacuum and purged with nitrogen.

To this mixture was added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II), dichloromethane complex (19 mg, 0.023 mmol), followed by degassing and purging with nitrogen. The reaction mixture was heated at 85 °C for 16 hours with efficient stirring. The reaction mixture was partitioned between EtOAc and aqueous NH_4OAc 25%w/v. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by chromatography using EtOAc and hexane as eluant to give the title compound as a colorless gum.

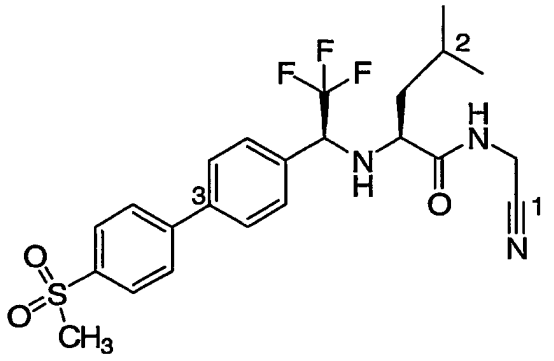
Step 6: *N*^{1}-(Cyanomethyl)-*N*^{2}-{[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl][4-(methylsulfonyl)phenyl]methyl}-L-Leucinamide

To a solution of *N*^{1}-(cyanomethyl)-*N*^{2}-{[4-(methylsulfonyl)phenyl][4'-(methylthio)-1,1'-biphenyl-4-yl]methyl}-L-leucinamide (63 mg, 0.12 mmol), sodium tungstate dihydrate (2 mg, 0.006 mmol), tetrabutylammonium hydrogensulfate (4 mg, 0.01 mmol) was added a solution of 30% w/v aqueous hydrogen peroxide (100 µL, 0.9 mmol) and the resulting mixture was stirred at room temperature for 10 min. The reaction mixture was partitioned between EtOAc and water + 1M $NaHSO_3$ (~3:1). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by chromatography using EtOAc and hexane as eluant to give the title compound as a colorless gum.

MS (+ESI): 568.2 $[M+1]^+$.

EXAMPLE 4

Synthesis of N^1(cyanomethyl)-N^2{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucinamide



Step 1: (2*S*)-1-{[*tert*-butyl(dimethyl)silyl]oxy}-4-methylpentan-2-amine

To a room temperature dichloromethane (100 mL) solution of L-leucinol (6.0 g) was added triethylamine (11 mL), DMAP (0.1 g) and t-butyldimethylsilyl chloride (8.5 g). The mixture was stirred at room temperature for 2 hours and then water was added. The organic layer was separated and the aqueous further extracted with dichloromethane. The combined organic layers were washed with brine, dried with magnesium sulfate and the solvent was removed in vacuo to yield the title compound, a residue which was used as such in the next reaction. ^{1}H NMR (CD_3COCD_3) δ 3.48(m, 2H), 3.32(m, 1H), 2.76(m, 1H), 1.78(m, 1H), 1.22-1.02(m, 2H), 0.88(m, 15H), 0.06(s, 6H).

Step 2: (2*S*)-1-{[*tert*-butyl(dimethyl)silyl]oxy}-4-methyl-*N*-[(1*E*)-2,2,2-trifluoroethylidene]pentan-2-amine

A toluene (300 mL) solution of (2*S*)-1-{[*tert*-butyl(dimethyl)silyl]oxy}-4-methylpentan-2-amine from Step 1 (50 g) and tifluoroacetaldehyde methyl hemiacetal (35 mL) was heated to reflux for 16 hours during which time water was collected in a Dean-Stark trap. The solvent was evaporated in vacuum and the residue was purified on SiO_2 using hexanes and ethyl acetate (9:1) as eluant to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 7.88(m, 1H), 3.76-3.45(m, 3H), 1.60-1.25(m, 3H), 0.88(m, 15H), 0.06(s, 3H), 0.04(s, 3H).

Step 3: (2S)-2-{[(1S)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]amino}-4-methylpentan-1-ol

n-BuLi (2.5 M in hexanes, 42 mL) was added to a –70 °C THF (400 mL) solution of 1,4-dibromobenzene (25.8 g) and the mixture was stirred for 25 minutes. A THF (30 mL) solution of (2*S*)-1-{[*tert*-butyl(dimethyl)silyl]oxy}-4-methyl-*N*-[(1*E*)-2,2,2-trifluoroethylidene]pentan-2-amine (31 g) was then added dropwise and the mixture was stirred for 1.5 hour. It was then poured slowly into a mixture of ethyl acetate (500 mL), water (2 L), ice (300 g) and ammonium chloride (100 g) under vigorous stirring. The organic layer was separated and the aqueous further extracted with ethyl acetate (2 X 500 mL). The combined organic layers were washed with brine, dried with magnesium sulfate and the solvent was removed in vacuo to yield a residue, which was used as such. The residue from above was dissolved in THF (250 mL) and the solution was cooled to 0 °C. A 1 M THF solution of t-butylammonium fluoride (110 mL) was added dropwise and the mixture was reacted for 4 hours. It was poured into ethyl acetate (300 mL), water (2 L) and ammonium chloride (100 g) under vigorous stirring. The organic layer was separated and the aqueous further extracted with ethyl acetate (2 X 100 mL). The combined organic layers were washed with brine, dried with magnesium sulfate and the solvent was removed in vacuo to yield a residue which was purified purified on SiO_2 using a gradient of ethyl acetate and hexanes (1:5 to 1:4) as eluant to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 7.6(2H, d), 7.45(2H, d), 4.55(1H, m), 3.65-3.7(1H, m), 3.5-3.55(1H, m), 3.25-3.35(1H, m), 2.6-2.7(1H, m), 2.25-2.35(1H, m), 1.65-1.75(1H, m), 1.3-1.4(1H, m), 1.2-1.3(1H, m), 0.75-0.9(6H, dd).

Step 4: (2S)-4-methyl-2-({(1S)-2,2,2-trifluoro-1-[4'-(methylthio)-1,1'-biphenyl-4-yl]ethyl}amino)pentan-1-ol

A stream of nitrogen was passed through a suspension made of the bromide from Step 3 (27.7 g), 4-(methylthio)phenylboronic acid (15.7 g), 2 M Na_2CO_3 (100 mL) and n-propanol (500 mL) for 15 minutes. A 1:3 mixture (3.5 g) of $Pd(OAc)_2$ and PPh_3 was then added and the reaction was warmed to 70 ^{0}C and stirred under nitrogen for 8 hours. The mixture was cooled to room temperature, diluted with ethylacetate (500 mL) and poured over water (2 L) and ice (500 g). The ethyl acetate layer was separated and the aqueous further extracted with ethyl acetate (200 mL). The combined ethyl acetate extracts were washed with 0.5 N NaOH (2 X 200 mL), with aqueous NH_4Cl, brine and dried with magnesium sulfate. Removal of the solvent left a residue that was purified by chromatography on SiO_2 using a gradient of ethyl acetate and hexanes (1:4 to 1:3) and again with acetone and toluene (1:10). The residue was dissolve in hot hexanes (200 mL) and the solution was allowed to cool to 0 ^{0}C under stirring. The obtained solid was filtered and dried to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 7.7(2H, d), 7.65(2H, d), 7.6(2H, d), 7.35(2H, d), 4.5-4.6(1H, m), 3.7(1H(OH), m), 3.5-3.6(1H, m), 3.3-3.4(1H, m), 2.7(1H, m), 2.5(3H, s), 2.3-2.4(1H(NH), m), 1.65-1.75(1H, m), 1.2-1.4(3H, m), 0.8-0.9(6H, dd).

Step 5: (2S)-4-methyl-2-({(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}amino)pentan-1-ol

To a 0 ^{0}C solution of the sulfide (19 g) from Step 4 in toluene (400 mL) was added $Na_2WO_4 \bullet 2H_2O$ (0.16 g) and Bu_4NHSO_4 (0.81 g). Then 30 % hydrogen peroxide (12.2 mL) was slowly added and the mixture was stirred at room temperature for 4.5 hours. The mixture was poured slowly on a mixture of ice, dilute aqueous sodium thiosulfate and ethyl acetate. The organic layer was separated and the aqueous further extracted with ethyl acetate (2 X 100 mL). The combined organic layers were washed with brine, dried with magnesium sulfate and the solvent were removed in vacuo to yield a residue which was purified purified on SiO_2 using ethyl acetate and hexanes (1:1) as eluant to yield the product.

^{1}H NMR (CD_3COCD_3) δ 8.05(2H, d), 8.0(2H, d), 7.85(2H, d), 7.7(2H, d), 4.6-4.7(1H, m), 3.75(1H, m), 3.6(1H, m), 3.35-3.45(1H, m), 3.2(3H, s), 2.7-2.8(1H, m), 2.35-2.45(1H, m), 1.7-1.8(1H, m), 1.2-1.5(2H, m), 0.8-0.95(6H, dd).

Step 6: Preparation of N-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucine

A suspension of H_5IO_6 /CrO_3 (529 mL of 0.44 M in CH3CN; see Note below) was cooled to 0 °C and a solution of the alcohol from Step 5 (20 g) in CH_3CN (230 mL) was added dropwise. The mixture was stirred at 0-5 °C for 3.5 hours. It was poured into pH 4 Na_2HPO_4 (1.5 L) under vigorous stirring and the mixture was extracted with diethyl ether (3 X 250 mL). The combined ether extracts were washed with water and brine (1:1), with dilute aqueous $NaHSO_3$ and brine. The organic extract was dried with sodium sulfate, filtered and the solvents were evaporated to dryness to yield a residue that was split into two batches for the following purification.

The crude acid from above (10 g) was dissolved in isopropyl acetate (250 mL) and extracted into cold 0.1 N NaOH (3 X 250 mL). The combined extracts were washed with diethyl ether (250 mL) and then slowly acidified with 6 N HCl to pH 4. The carboxylic acid was extracted with isopropyl acetate (2 X 250 mL) and the isopropyl acetate layer dried and concentrated to yield the product essentially pure and used as such in the next step.

Note: The oxidizing reagent (H_5IO_6 /CrO_3) was prepared as described in Tetrahedron Letters 39 (1998) 5323-5326 but using HPLC grade CH_3CN (contains 0.5% water); no water was added.

^{1}H NMR (CD_3COCD_3) δ 8.05(2H, d), 7.95(2H, d), 7.8(2H, d), 7.65(2H, d), 4.45-4.55(1H, m), 3.55-3.6(1H, m), 3.2(3H, s), 2.8-3.0(broad m, NH/OH)1.95-2.05(1H, m), 1.55-1.6(2H, m), 0.9-1.0(6H, m).

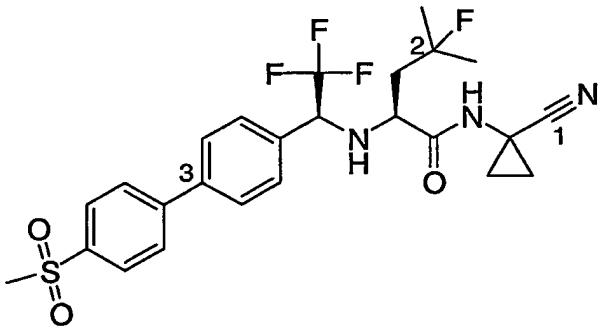
Step 7: Preparation of N^1(cyanomethyl)-N^2-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucinamide

To a DMF (200 mL) solution of the acid from Step 7 (9 g) was added benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (11.6 g), aminoacetonitrile hydrochloride (3.94 g) and the mixture was cooled to 0 °C. Triethylamine (9.9 mL) was added dropwise and the mixture warmed to room temperature and stirred for 16 hours. It was poured into ice and saturated aqueous sodium bicarbonate and extracted with diethyl ether (3 X 100 mL). The combined extracts were washed with brine, dried with magnesium sulfate and the solvent removed in vacuo. The residue was purified by chromatography on SiO_2 using ethyl acetate and hexanes (1:1). The title compound was then stirred in diethyl ether for 16 hours, filtered and dried (mp 140.5 °C).

^{1}H NMR (CD_3COCD_3) δ 8.0(2H, d), 7.95(2H, d), 7.8(2H, d), 7.65(2H, d), 4.35-4.45(1H, m), 4.1-4.2(2H, m), 3.45-3.55(1H, m), 3.15(3H, s), 2.65-2.7(1H, m), 1.85-1.95(1H, m), 1.4-1.6(2H, m), 0.85-0.95(6H, m).

EXAMPLE 5

Preparation of *N*^{1}-(1-cyanocyclopropyl)-4-fluoro-*N*^{2}-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucinamide



Step 1: Benzyl (3*S*)-3-[(*tert*-butoxycarbonyl)amino]-4-hydroxybutanoate

N-(*tert*-Butoxycarbonyl)-L-aspartic acid 4-benzyl ester (30 g) was dissolved in dimethoxyethane (90 mL) and the solution was cooled to –5 °C. N-Methylmorpholine (10.32 mL) was added followed by isobutyl chloroformate (12.7 mL) in such a way to keep the temperature below –10 °C. The mixture was aged for 0.5 hour. The solids were quickly filtered and washed with dimethoxyethane (90 mL). To the filtrate cooled to – 50 °C was carefully added sodium borohydride (4.4 g) as a solution in water (45 mL) in such a way to keep the temperature between –30 °C and –15 °C. After all the hydride had been added, water (500 mL) was added in such a way to maintain the temperature below –15 °C. The suspension was filtered, the solid washed with water (400 mL) and dried to yield benzyl (3*S*)-3-[(*tert*-butoxycarbonyl)amino]-4-hydroxybutanoate.

^{1}H NMR (CD_3COCD_3) δ 7.3-7.45(5H, m), 5.85-5.95(1H, NH), 5.15(2H, s), 3.95-4.1(2H, m), 3.5-3.7(2H, m), 2.55-2.75(2H, m), 1.4(9H, s).

Step 2: Benzyl [(4*S*)-2-oxo-1,3-oxazolidin-4-yl]acetate

To the alcohol (95.7 g) from Step 1 dissolved in dichloroethane (925 mL) was added pyridine (625 mL) and the mixture was cooled to 0-5 °C. Anhydrous p-toluenesulfonic anhydride (105.7 g.) was added and the mixture was warmed to room temperature and stirred for 1 hour. It was then heated

to 90 °C for 2 hours. The mixture was cooled, diluted with dichloromethane (1000 mL) and washed with 1N HCl (3 X 600 mL). The organic layer was washed with brine, dried with sodium sulfate and the solvents were removed *in vacuo*. The residue was purified by chromatography on SiO_2 using ethyl acetate and hexanes in a 1:1 ratio followed by ethyl acetate to yield benzyl [(4*S*)-2-oxo-1,3-oxazolidin-4-yl]acetate.

^{1}H NMR (CD_3SOCD_3) δ 7.8(1H, NH), 7.3-7.45(5H, m), 5.05-5.15(2H, m), 4.4-4.5(1H, m), 4.1-4.2(1H, m), 4.0-4.05(1H, m), 3.6-3.8(2H, m).

Step 3: (4*S*)-4-(2-Hydroxy-2-methylpropyl)-1,3-oxazolidin-2-one.

Methylmagnesium bromide (227 mL of 3M solution in diethyl ether) was added to a mixture of toluene (340 mL) and THF (340 mL) at –20 °C. The ester from Step 2 (40 g) as a warm THF solution (170 mL) was then added dropwise maintaining the temperature below –10 °C and the mixture was aged for 2 hours. The mixture was then slowly added to a mixture of water (1000 mL) and acetic acid (200 mL) and the mixture was stirred for 2 hours at room temperature. The aqueous layer was separated and the organic extracted with water (2 X 200 mL). The product was extracted from the combined aqueous layers using dichloromethane and a continuous extractor. The dichloromethane extract was evaporated to dryness with the help of heptane. The residue was purified by chromatography on SiO_2 using ethanol and dichloromethane (1:30) to yield (4*S*)-4-(2-hydroxy-2-methylpropyl)-1,3-oxazolidin-2-one.

^{1}H NMR (CD_3COCD_3) δ 6.1-6.4(1H, NH), 4.45-4.55(1H, m), 4.1-4.2(1H, m), 3.95-4.05(1H, m), 3.7(1H, s), 1.65-1.85(2H, m), 1.25(6H, m).

Step 4: (4*S*)-4-(2-Fluoro-2-methylpropyl)-1,3-oxazolidin-2-one.

The alcohol (47.8 g.) from Step 3 as a dichloromethane (100 mL) solution was added to a -70 °C solution of (diethylamino)sulfur trifluoride (48.5 g.) in dichloromethane (500 mL). The mixture was warmed to room temperature and stirred for 1 hour. It was then carefully added to a 0 °C mixture of saturated aqueous $NaHCO_3$ (800 mL). The organic layer was separated and washed with saturated aqueous $NaHCO_3$. The aqueous was further extracted with dichloromethane (100 mL) and the combined dichloromethane layers were dried and concentrated. The residue was purified by chromatography on SiO_2 using ethyl acetate and hexanes (1:5) followed by ethyl acetate to yield (4*S*)-4-(2-fluoro-2-methylpropyl)-1,3-oxazolidin-2-one.

^{1}H NMR (CD_3SOCD_3) δ 7.6(1H, NH), 4.4-4.5(1H, m), 3.95-4.05(1H, m), 3.9-3.95(1H, m), 1.8-1.95(2H, m), 1.25-1.4(6H, 2s).

Step 5: (2*S*)-2-Amino-4-fluoro-4-methylpentan-1-ol.

To the fluoro derivative (21.0 g) from Step 4 dissolved in 90% aqueous ethyl alcohol (216 mL) was added potassium hydroxide (21.9 g). The mixture was heated at reflux for 4 hours and cooled to room temperature. The mixture was then concentrated and co-evaporated with toluene (3 X 300 mL). The residue was dissolved in dichloromethane (500 mL) and stirred for 0.5 hour. The suspension was filtered through celite and the celite was washed with dichloromethane (3 X 100 mL). The filtrate was concentrated to dryness to yield (2*S*)-2-amino-4-fluoro-4-methylpentan-1-ol.

^{1}H NMR (CD_3OD) δ 3.4-3.5(1H, m), 3.2-3.3(1H, m), 3.0-3.1(1H, m), 1.5-1.7(2H, m), 1.35(3H, s), 1.3(3H, s).

Step 6: (2S)-1-{[*tert*-butyl(dimethyl)silyl]oxy}-4-fluoro-4-methylpentan-2-amine

The amino alcohol (21.0 g) from Step 5 was dissolved in dichloromethane (300 mL) and the solution was cooled to 0 °C. 4-(Dimethylamino)pyridine (0.051 g.) and *tert*-butyldimethylsilyl chloride (21 g.) were added followed by triethylamine (25 mL). The mixture was stirred at room temperature overnight. The reaction mixture was slowly poured into 0 °C saturated aqueous ammonium chloride and extracted with dichloromethane (3 X 300 mL). The organic layer was washed with brine, dried with sodium sulfate and the solvents were removed *in vacuo* to yield (2S)-1-{[*tert*-butyl(dimethyl)silyl]oxy}-4-fluoro-4-methylpentan-2-amine.

^{1}H NMR (CD_3OD) δ 3.6-3.65(1H, m), 3.4-3.5(1H, m), 3.1-3.2(1H, m), 1.6-1.8(2H, m), 1.35-1.45(6H, m), 0.93(9H, s), 0.1(6H, s).

Step 7: (2*S*)-1-{[*tert*-butyl(dimethyl)silyl]oxy}-4-fluoro-4-methyl-*N*-[(1*E*)-2,2,2-trifluoroethylidene]pentan-2-amine.

To the amine (31.5 g) from Step 6 dissolved in benzene (126 mL) was added trifluoroacetaldehyde methyl hemiacetal (21.6 mL.). The solution was heated at reflux overnight using a Dean-Stark trap to collect water. The reaction mixture was cooled to room temperature and concentrated to dryness. The residue was purified on SiO_2 using 4% of ethyl acetate in hexanes to yield (2S)-1-{[*tert*-butyl(dimethyl)silyl]oxy}-4-fluoro-4-methylpentan-2-amine.

^{1}H NMR (CD_3COCD_3) δ 7.9-7.95(1H, m), 3.75-3.85(1H, m), 3.7-3.75(1H, m), 3.53-3.6(1H, m), 1.9-2.0(2H, m), 1.3-1.4(6H, m), 0.9(9H, s), 0.1(3H, s), 0.05(3H, s).

Step 8: (2*S*)-2-{[(1*S*)-1-(4-Bromophenyl)-2,2,2-trifluoroethyl]amino}-4-fluoro-4-methylpentan-1-ol.

To a –75 °C solution of 1,4-dibromobenzene (0.26 g) in THF (4 mL) was added n-BuLi (0.42 mL of a 2.5M hexanes solution) and the mixture was aged for 20 minutes. The imine (0.329 g.) from Step 7 in THF (2 mL) was added and the mixture was aged 2 hours. The mixture was then added to a mixture of water (50 mL), NH_4Cl (1 g.) and crushed ice. It was extracted with ethyl acetate (2 X 25 mL) and the combined ethyl acetate layers were dried and evaporated to dryness.

The same procedure was repeated but using dibromobenzene (1.2 g.), n-BuLi (1.84 mL) and the imine (1.38 g.) and the reaction mixture was treated as above. The combined residues from both preparations were dissolved in THF (10 mL) and cooled to 0 °C. n-Tetrabutylammonium fluroride (6 mL from a 1M THF solution) was added and the mixture was stirred at + 5 °C for 16 hrs. It was poured into a mixture of water (50 mL), ammonium chloride (1 g.) and crushed ice and the organic layer was separated. The aqueous was further extracted with ethyl acetate (2X 15 mL) and the combined organic layers were dried and concentrated. The residue was purified on SiO_2 using ethyl acetate and hexanes (1:5) to yield (2*S*)-2-{[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]amino}-4-fluoro-4-methylpentan-1-ol.

^{1}H NMR (CD_3COCD_3) δ 7.65(2H, m), 7.5(2H, m), 4.5-4.6(1H, m), 3.8(1H, m), 3.6(1H, m), 3.3-3.4(1H, m), 2.85-2.0(1H, m), 2.55(1H, m), 1.7-1.9(2H, s), 1.3-1.4(6H, m).

Step 9: *N*-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-4-fluoro-L-leucine.

A suspension of H_5IO_6 /CrO_3 (66 mL of 0.44 M in CH_3CN; *Note*) was cooled to 0 °C and a solution of the alcohol from Step 8 (1.55 g) in CH_3CN (5 mL) was added dropwise. The mixture was stirred at 0-5 °C for 3.5 hours. It was poured into pH 4 Na_2HPO_4 (200 mL) under vigorous stirring and the mixture was extracted with diethyl ether (3 X 50 mL). The combined ether extracts were washed with water and brine (1:1) followed by dilute aqueous $NaHSO_3$ and brine. It was dried with sodium sulfate, filtered and the solvents were evaporated to dryness to yield of *N*-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-4-fluoro-L-leucine used as such in the next step.

Note. The oxidizing reagent (H_5IO_6 /CrO_3) was prepared as described in Tetrahedron Letters 39 (1998) 5323-5326 but using HPLC grade CH_3CN (contains 0.5% water); no water was added.

Step 10: N^2-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(1-cyanocyclopropyl)-4-fluoro-L-leucinamide.

Diisopropylethylamine (4.2 mL) was added to a 0 °C suspension of the acid (1.5 g) from Step 9, 1-amino-1-cyclopropanecarbonitrile hydrochloride (1.18 g), *O*-(7-azabenzotriazol-1-yl)-*N, N, N', N'*-tetramethyluronium hexafluorophosphate (1.94 g) and dimethylformamide (5 mL) and the mixture was reacted at room temperature for 48 hrs. It was then poured on ice and dilute aqueous ammonium chloride. The mixture was extracted with ethyl acetate and ether (1:1) and the combined organic layers were washed with pH 3 dilute Na_2HPO_4 and brine. The solvents were evaporated to dryness and the residue was purified by chromatography on SiO_2 using ethyl acetate and hexanes (1:2) to yield N^2-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(1-cyanocyclopropyl)-4-fluoro-L-leucinamide in a sufficient purity state for the next step.

^{1}H NMR (CD_3COCD_3) δ 8.15(1H, NH), 7.6(2H, m), 7.45(2H, m), 4.35-4.45(1H, m), 3.45-3.55(1H, m), 1.9-2.1(2H, m), 1.75-1.85(1H, NH),1.35-1.55(8H, m), 1.1-1.15(1H, m), 0.95-1.05(1H, m).

Step 11: N^1-(1-cyanocyclopropyl)-4-fluoro-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylthio)-1,1'-biphenyl-4-yl]ethyl}-L-leucinamide.

A stream of nitrogen was passed through a suspension made of the bromide from Step 10 (0.338 g.), 4-(methylthio)phenylboronic acid (0.252 g), 2M Na_2CO_3 (0.8 mL) and DMF (4 mL) for 15 minutes. $PdCl_2 \cdot$ dppf (0.1 g) was then added and the reaction was warmed to 85 °C and stirred under nitrogen for 5 hours. The mixture was cooled to room temperature, diluted with ethyl acetate (10 mL) and poured into water (50 mL) and ice. The ethyl acetate layer was separated and the aqueous further extracted with ethyl acetate. The combined ethyl acetate extracts were dried and the solvents removed *in vacuo*. The residue was purified by chromatography on SiO_2 using ethyl acetate and hexanes (1:2) to yield N^1-(1-cyanocyclopropyl)-4-fluoro-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylthio)-1,1'-biphenyl-4-yl]ethyl}-L-leucinamide.

^{1}H NMR (CD_3COCD_3) δ 8.15(1H, NH), 7.1-7.2(4H, m),7.5-7.55(2H, m), 7.35-7.4(2H, m), 4.3-4.4(1H, m), 3.45-3.55(1H, m), 2.75-2.8(1H, NH), 2.5(3H, s), 1.9-2.05(2H, m), 1.3-1.5(8H, m), 1.0-1.1(1H, m), 0.85-0.95(1H, m).

Step 12: Preparation of N^1-(1-cyanocyclopropyl)-4-fluoro-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucinamide

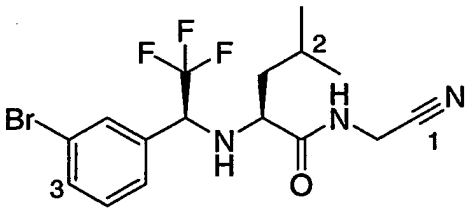
To a 0 ° solution of the sulfide (0.265 g) from Step 11 in toluene (5 mL) and dichloromethane (5 mL) was added $Na_2WO_4 \bullet 2H_2O$ (0.002 g) and n-Bu_4NHSO_4 (0.01 g). Then 30 % hydrogen peroxide (0.137 mL) was slowly added and the mixture was stirred at room temperature for 3

hours. The mixture was poured slowly on a mixture of ice, dilute aqueous sodium thiosulfate and ethyl acetate. . The organic layer was separated and the aqueous further extracted with ethyl acetate. The combined organic layers were washed with brine, dried with magnesium sulfate and the solvent were removed *in vacuo* to yield a residue which was purified on SiO_2 using ethyl acetate, hexanes and dichloromethane (1:1:0.1) as eluant. The residue was triturated in diethyl ether to yield N^1-(1-cyanocyclopropyl)-4-fluoro-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucinamide.

^{1}H NMR (CD_3COCD_3) δ 8.2(1H, NH), 8.05-8.1(2H, m), 7.95-8.0(2H, m), 7.8(2H, m), 7.65(2H, m), 4.35-4.45(1H, m), 3.5-3.6(1H, m), 3.2(3H, s), 2.8-2.9(1H, NH), 1.9-2.1(2H, m), 1.3-1.5(8H, m), 1.05-1.15(1H, m), 0.9-1.0(1H, m).

EXAMPLE 6

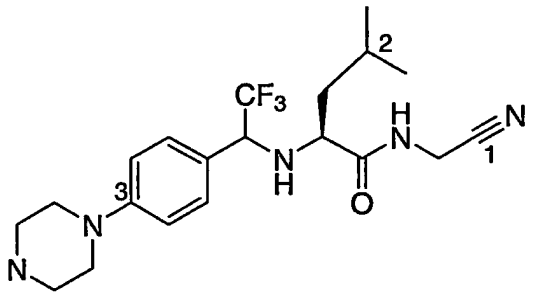
Synthesis of N^2-[1-(3-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(cyanomethyl)-L-leucinamide



Using the procedure described for Example 1, where 2,2,2-trifluoroacetophenone was substituted for 1-(3-bromophenyl)-2,2,2-trifluoroethanone, the title compound was prepared.
MS (+ESI): 406.0, 408.1 $[M+1]^+$.

EXAMPLE 7

N^1-(Cyanomethyl)-N^2-[2,2,2-trifluoro-1-(4-piperazin-1-ylphenyl)ethyl]-L-leucinamide



Step 1: N^2-(1-{4-[4-(*tert*-butylcarboxylate)piperazin-1-yl]phenyl}-2,2,2-trifluoroethyl)-N^1-(cyanomethyl)-L-leucinamide

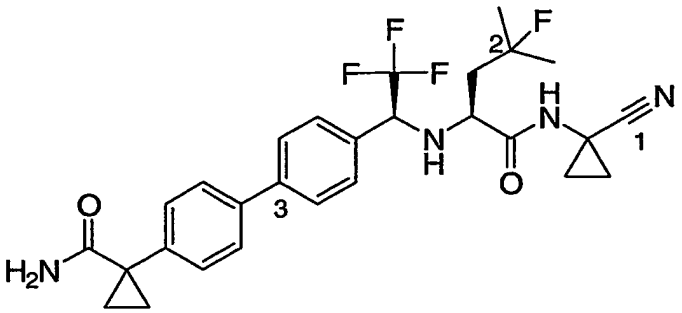
A solution of N^2-[1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N1-(cyanomethyl)-L-leucinamide (example 2) (100 mg, 0.25 mmol), tris(dibenzylideneacetone)dipalladium (2.3mg, 0.0025mmol), biphenyl-2-yl(di-*tert*-butyl)phosphine (3mg, 0.01 mmol), potassium phosphate (74 mg, 0.35 mmol) and tert-butyl piperazine-1-carboxylate (56 mg, 0.3 mmol) in dimethoxyethane (0.5 mL) was cooled to –78 °C pumped under high vacuum for 3 minutes, then nitrogen was admitted in the flask. The mixture was then heated at 80 °C for 1 hr. After cooling to room temperature, the mixture was applied directly on a silica gel column and eluted with ethyl acetate/ hexane to afford the title compound.

Step 2: N^1-(Cyanomethyl)-N^2-[2,2,2-trifluoro-1-(4-piperazin-1-ylphenyl)ethyl]-L-leucinamide.

To the compound from step 1 (139 mg, 0.27 mmol) in 1,4-dioxane (0.5 mL) was added methanesulfonic acid (53 uL, 0.82mmol) and the mixture was stirred over night. Ethyl acetate was added, then the mixture was washed with saturated sodium bicarbonate, brine, dried over magnesium sulfate, filtered and the solvent evaporated under vacuum. Purification by silica gel chromatography eluting with 93% dichloromethane, 0.6% ammonium hydroxide and 6.4% methanol afforded the title compound.

MS (+ESI): 412.2 $[M+1]^+$.

EXAMPLE 8



Synthesis of N^2-((1*S*)-1-{4'-[1-(aminocarbonyl)cyclopropyl]biphenyl-4-yl}-2,2,2-trifluoroethyl)-N^1-(1-cyanocyclopropyl)-4-fluoro-L-leucinamide

Step 1: Preparation of 1-(4-bromophenyl)cyclopropanecarbonitrile

To a room temperature solution of 4-bromophenylacetonitrile (18.0 g) in 22 mL of sodium hydroxide (50% in water W/W) were added 1-bromo-2-chloroethane and (12.0 mL) and benzyltrimethylammonium chloride (627 mg). The mixture was heated at 60 °C overnight. The reaction mixture was cooled to room temperature and diethyl ether was added (300 mL. The ether layer was washed with water (100 mL), hydrogen chloride (100 mL, 10% HCl in water) and brine. The organic layer was dried with magnesium sulfate and the solvent removed in vacuo. The residue was purified by trituration using diethyl ether and hexanes to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 7.60(2H, d), 7.35(2H, d), 1.74-1.80(2H, m), 1.52-1.57(2H, m).

Step 2 : Preparation of 1-(4-bromophenyl)cyclopropanecarboxylic acid

To a room temperature solution of 1-(4-bromophenyl)cyclopropanecarbonitrile from Step 1 (13 g) in ethyl alcohol (110 mL) was added a solution of 56 mL of sodium hydroxide (25% NaOH in water W/W). The mixture was heated at 100 °C overnight. It was cooled to room temperature, poured into ice and hydrogen chloride (1 N) and extracted with dichloromethane (2 X 100 mL). The combined extracts were washed with brine, dried with magnesium sulfate and the solvent removed in vacuo to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 7.50(2H, d), 7.35(2H, d), 1.53-1.60(2H, m), 1.18-1.22(2H, m).

Step 3: Preparation of 1-(4-bromophenyl)cyclopropanecarboxamide

To a –15 °C solution of 1-(4-bromophenyl)cyclopropanecarboxylic acid from Step 2 (1.5 g) in chloroform (60 mL) were slowly added isobutyl chloroformate (900 µL) and triethylamine (1.1 mL). The reaction mixture was stirred at –15 °C for 2 hours. Then it was saturated with ammonia gas and stirred at -15 °C for 10 minutes. The reaction mixture was allowed to stand at room temperature for 1 hour then poured into water (60 mL) and partitioned. The aqueous layer was extracted with dichloromethane (2 X 60 mL). The combined extracts were washed with brine, dried with magnesium sulfate and the solvent removed in vacuo. The residue was purified by swish using diethyl ether and hexanes to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 7.54(2H, d), 7.40(2H, d), 6.45(1H, bs), 5.96(1H, bs), 1.42-1.48(2H, m), 0.98-1.02(2H, m).

Step 4: Preparation of N^1-(1-cyanocyclopropyl)-4-fluoro-N^2-{(1*S*)-2,2,2-trifluoro-1-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]ethyl}-L-leucinamide

A stream of nitrogen was passed through a DMF (40 mL) suspension of N^2-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(1-cyanocyclopropyl)-4-fluoro-L-leucinamide from Example 5, Step 9 (2.0 g), bis(pinacolato)diboron (1.24 g) and potassium acetate (1.53 g) for 15 minutes. The catalyst [1, 1'-bis(diphenylphosphino)-ferrocene]dichloropalladium(II), complex (1:1) with dichloromethane (181 mg) was then added and the mixture warmed to 65 ^{0}C overnight under nitrogen. The mixture was cooled to room temperature, diluted with ethyl acetate and hexanes (1:1, 100 mL) and poured over water (50 mL) and ice (50 g). The organic layer was separated and the aqueous layer further extracted with ethyl acetate and hexanes (1:1, 3 X 50 mL). The combined extracts were washed with brine and dried with magnesium sulfate. Removal of the solvent left a residue which was purified by chromatography on SiO_2 using ethyl acetate and hexanes (1:3 to 1:2) to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 8.15(1H, bs), 7.78(2H, d), 7.50(2H, d), 4.31-4.40 (1H, m), 3.47-3.54 (1H, m), 2.72-2.80 (2H, m), 1.32-1.48(9H, m), 1.05-1.11(1H, m), 0.87-0.95(1H, m).

Step 5: Preparation of N^2-((1*S*)-1-{4'-[1-(aminocarbonyl)cyclopropyl]biphenyl-4-yl}-2,2,2-trifluoroethyl)-N^1-(1-cyanocyclopropyl)-4-fluoro-L-leucinamide

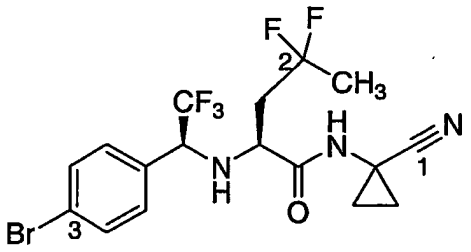
A stream of nitrogen was passed through a solution of DMF (4 mL) of the boronate from Step 4 (150 mg), 1-(4-bromophenyl)cyclopropanecarboxamide from Step 3 (110 mg) and 2 M Na_2CO_3 (400 µL) for 15 minutes. The catalyst [1, 1'-bis(diphenylphosphino)-ferrocene]dichloropalladium(II), complex (1:1) with dichloromethane (12 mg) was then added and the mixture was warmed to 80 ^{0}C for 3 hours under nitrogen. The mixture was cooled to room temperature, poured into ice (10 g) and saturated

aqueous sodium bicarbonate (20 mL) and extracted with 50 % ethyl acetate (3 X 30 mL). The combined extracts were washed with brine and dried with magnesium sulfate. Removal of the solvent left a residue which was purified by chromatography on SiO_2 using ethyl acetate and hexanes (50 to 70%) as eluants, followed by a swish using diethyl ether to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 8.20(1H, bs), 7.75(2H, d), 7.70(2H, d), 7.60(2H, d), 7.55(2H, d), 6.37(1H, bs), 5.87(1H, bs), 4.35-4.43 (1H, m), 3.52-3.58 (1H, m), 1.92-2.05 (2H, m), 1.42-1.50(6H, m), 1.35-1.42(4H, m), 1.03-1.12(3H, m), 0.92-0.98(1H, m).

EXAMPLE 9

N^2-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-*N*^{1}-(1-cyanocyclopropyl)-4,4-difluoro-L-norvalinamide



Step 1: Preparation of methyl *N*-((benzyloxy)carbonyl)-3-iodo-L-alaninate

To a solution of carbobenzyloxy-L-serine (25 g, 104 mmol) in ethyl acetate (200 mL) was added a solution of diazomethane in ether until a slight yellow color persisted. The solvent was evaporated under vacuum. To the residue was added N,N-dimethylformamide (400 mL) and methyltriphenoxyphosphonium iodide (50 g, 110 mmol). The mixture was stirred for 15 minutes, then methanol (15 mL) was added and the mixture was then poured over 20 % sodium thiosulfate and extracted with a 1:1 mixture of ethyl acetate:hexanes (2 L). The organic layer was washed with water, brine (3 x), dried over magnesium sulfate, filtered and the solvent evaporated under vacuum. The residue was purified by silica gel chromatography using ethyl acetate and hexanes. The compound obtained was triturated in diethyl ether/hexanes, filtered and air dried to afford methyl *N*-((benzyloxy)carbonyl)-3-iodo-L-alaninate.

Step 2: Preparation of methyl *N*-((benzyloxy)carbonyl)-4-oxo-L-norvalinate

A mixture of methyl *N*-((benzyloxy)carbonyl)-3-iodo-L-alaninate (10 g, 27.5 mmol), from Step 1, zinc-copper couple (3.3 g) in benzene (110 mL) and N,N-dimethylacetamide (7.4 mL) was sonicated in an ultra-sound bath for 2 hours. Over this period, 3 portions of 1,2-dibromoethane (0.24

mL) and chlorotrimethylsilane (0.17 mL) were added. To this mixture was then added bis(triphenylphosphine)palladium chloride (0.958 g, 1.4 mmol) and acetyl chloride (2.5 mL, 35.2 mmol) and the mixture was heated at 70 ^{0}C for 2 hours. After cooling to room temperature, the mixture was filtered on celite with ethyl acetate, the organic layer was then washed with a saturated solution of ammonium chloride, brine (2x), dried over magnesium sulfate, filtered and the solvent evaporated under vacuum. The residue was purified by silica gel chromatography using ethyl acetate and hexanes to afford methyl *N*-((benzyloxy)carbonyl)-4-oxo-L-norvalinate.

Step 3: Preparation of methyl *N*-((benzyloxy)carbonyl)-4,4-difluoro-L-norvalinate

To a solution of methyl *N*-((benzyloxy)carbonyl)-4-oxo-L-norvalinate (1.3 g, 4.65 mmol) in dichloromethane (20 mL) and methanol (0.019 mL) at 0 ^{0}C was added DAST (2.46 mL) slowly. The ice bath was removed and replaced with a hot water (57 ^{0}C) bath. The hot water bath was replaced 3 times, then the mixture was stirred overnight at room temperature. The mixture was slowly poured over cold saturated $NaHCO_3$, extracted with ethyl acetate, washed with brine, dried over magnesium sulfate, filtered and the solvent evaporated under vacuum. The residue was purified by silica gel chromatography using ethyl acetate and hexanes to afford methyl *N*-((benzyloxy)carbonyl)-4,4-difluoro-L-norvalinate.

Step 4: Preparation of benzyl (1*S*)-3,3-difluoro-1-(hydroxymethyl)butylcarbamate

To a solution of methyl *N*-((benzyloxy)carbonyl)-4,4-difluoro-L-norvalinate (1.59 g, 5.29 mmol) in ethanol (50 mL) was added lithium chloride (919 mg) and the mixture was stirred for 10 minutes. Sodium borohydride (820 mg) was added slowly, the mixture stirred for 2 hours. Then, another portion of sodium borohydride (100 mg) was added and stirring continued for 30 minutes. The mixture was diluted with water (20 mL) and neutralized slowly with 1N HCl followed by the addition of another aliquot of water. The mixture was extracted with ethyl acetate (2x), washed with brine, dried over magnesium sulfate, filtered and the solvent evaporated under vacuum to afford benzyl (1*S*)-3,3-difluoro-1-(hydroxymethyl)butylcarbamate.

Step 5: Preparation of (2*S*)-1-((*tert*-butyl(dimethyl)silyl)oxy)-4,4-difluoropentan-2-amine

To a solution of benzyl (1*S*)-3,3-difluoro-1-(hydroxymethyl)butylcarbamate (from Step 4) in ethanol (25 mL) was added palladium on charcoal (10 %, 150 mg) and the mixture was stirred under a H_2 atmosphere (ballon) for 2 h. Dichloromethane was added and the mixture was filtered on celite. The solvent was evaporated under vacuum. The residue was dissolved in dichloromethane (15 mL) and triethylamine (1 mL), N,N-dimethylaminopyridine (10 mg) and chloro-t-butyldimethylsilane (844 mg) were added. The mixture was stirred overnight, then water and brine were added. The mixture was extracted with ethyl acetate (2x), washed with brine, dried over magnesium sulfate, filtered and the

solvent evaporated under vacuum to afford (2*S*)-1-((*tert*-butyl(dimethyl)silyl)oxy)-4,4-difluoropentan-2-amine.

Step 6: Preparation of (2*S*)-1-((*tert*-butyl(dimethyl)silyl)oxy)-4,4-difluoro-*N*-((1*E*)-2,2,2-trifluoroethylidene)pentan-2-amine

A solution of (2*S*)-1-((*tert*-butyl(dimethyl)silyl)oxy)-4,4-difluoropentan-2-amine, from Step 5, and trifluoroacetaldehyde methyl hemiacetal (80 %, 0.9 mL) in benzene (20 mL) was refluxed over night with a Dean-Stark apparatus. The solvent was evaporated under vacuum and the residue purified by silica gel chromatography using ethyl acetate and hexanes to afford (2*S*)-1-((*tert*-butyl(dimethyl)silyl)oxy)-4,4-difluoro-*N*-((1*E*)-2,2,2-trifluoroethylidene)pentan-2-amine.

Step 7: Preparation of (2*S*)-2-(((1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl)amino)-4,4-difluoropentan-1-ol

To a –78 ^{0}C solution of 1,4-dibromobenzene (330 mg) in THF (5.2 mL) was added 2.5M n-BuLi in hexanes (0.52 mL) and the solution was aged for 30 minutes. Then, a solution of (2*S*)-1-((*tert*-butyl(dimethyl)silyl)oxy)-4,4-difluoro-*N*-((1*E*)-2,2,2-trifluoroethylidene)pentan-2-amine (333 mg) in THF (5.2 mL) was added. The mixture was stirred at –78 ^{0}C for 45 minutes, then poured over cold saturated ammonium chloride, extracted with ethyl acetate (2x), washed with brine, dried over magnesium sulfate, filtered and the solvent evaporated under vacuum. The residue was dissolved in THF (10 mL) cooled in an ice/water bath and n-tetrabutylammonium fluroride (1M in THF, 1.5 mL) was added. The mixture was stirred at 0 ^{0}C for 1 h, poured over cold water, extracted with ethyl acetate (2x), washed with brine, dried over magnesium sulfate, filtered and the solvent evaporated under vacuum to afford (2*S*)-2-(((1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl)amino)-4,4-difluoropentan-1-ol.

Step 8: Preparation of N^2-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(1-cyanocyclopropyl)-4,4-difluoro-L-norvalinamide

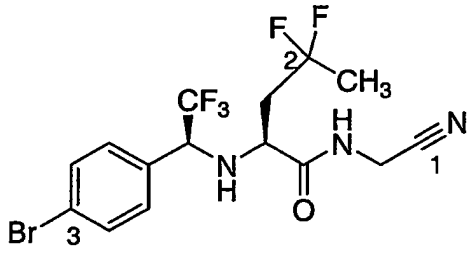
A suspension of H_5IO_6 /CrO_3 (27 mL of 0.44 M in CH_3CN; *Note*) was cooled to 0 ^{0}C and a solution of the alcohol from Step 7 (740 mg) in CH_3CN (10 mL) was added dropwise. The mixture was stirred at 0 ^{0}C for 4 hours, with addition of more H_5IO_6 /CrO_3 (2 x 10 mL of 0.44 M in CH_3CN). Then the mixture was poured into pH 4 Na_2HPO_4 buffer under vigorous stirring and the mixture was extracted with ethyl acetate washed with brine (2x) followed by dilute aqueous $NaHSO_3$ and brine. The mixture was dried with magnesium sulfate, filtered and the solvents were evaporated to dryness to yield 680 mg of *N*-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-4,4-difluoro-L-norvaline which was used as such in the next step.

Note. The oxidizing reagent (H_5IO_6 /CrO_3) was prepared as described in Tetrahedron Letters 39 (1998) 5323-5326 but using HPLC grade CH_3CN (contains 0.5% water); no water was added.

Triethylamine (0.42 mL) was added to mixture of the acid (340 mg) from above, 1-amino-1-cyclopropanecarbonitrile hydrochloride (227 mg), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (498 mg) and dimethylformamide (4.5 mL) and the mixture was reacted at room temperature for 48 h. It was then poured on dilute sodium bicarbonate. The mixture was extracted with ethyl ether (3x) and the combined organic layers were washed with brine (3x) and dried with magnesium sulfate filtered. The solvents were evaporated to dryness and the residue was purified by chromatography on silica gel using ethyl acetate 40 % and hexanes to yield N^2-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(1-cyanocyclopropyl)-4,4-difluoro-L-norvalinamide.
MS (+ESI): 454.1, 456.2 $[M+1]^+$.

EXAMPLE 10

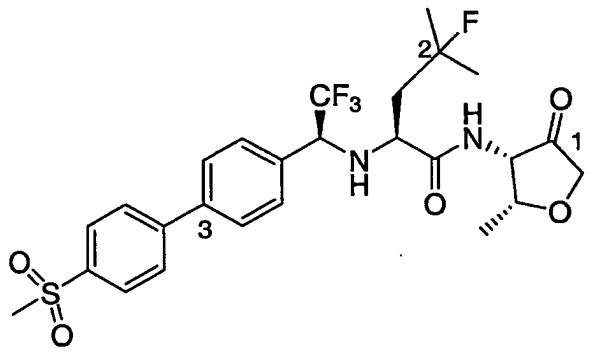
N^2-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(cyanomethyl)-4,4-difluoro-L-norvalinamide



Using the procedure described for Example 9, where 1-amino-1-cyclopropanecarbonitrile hydrochloride was substituted for aminoacetonitrile hydrochloride in Step 8, the title compound was obtained
MS (+ESI): 428, 430.1 $[M+1]^+$.

EXAMPLE 11

4-fluoro-N^1-[(2*R*,3*S*)-2-methyl-4-oxotetrahydrofuran-3-yl]-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide.



Step 1: N^2-[(1*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]-4-fluoro-N^1-[(2*R*,3*S*)-2-methyl-4-oxotetrahydrofuran-3-yl]-L-leucinamide

Triethylamine (0.63 mL) was added to mixture of the acid (500 mg) from Example 5, Step 9, (4*S*,5*R*)-4-amino-5-methyldihydrofuran-3(2*H*)-one hydrochloride (227 mg) (WO 00/69855), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (744 mg) and dimethylformamide (7 mL) and the mixture was reacted at room temperature for 3 hrs. It was then poured on dilute sodium bicarbonate. The mixture was extracted with ethyl ether (3x) and the combined organic layers were washed with brine (3x) and dried with magnesium sulfate filtered. The solvents were evaporated to dryness and the residue was purified by chromatography on silica gel using ethyl acetate 30 % and hexanes to yield the title compound.

Step 2: 4-fluoro-N^1-[(2*R*,3*S*)-2-methyl-4-oxotetrahydrofuran-3-yl]-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylthio)biphenyl-4-yl]ethyl}-L-leucinamide

A mixture of the bromide from Step 1 (200 mg), 4-(methylthio)phenylboronic acid (104 mg), 2M aqueous Na_2CO_3 (0.51 mL) and DMF (3 mL) for 15 minutes. $PdCl_2dppf_2$ (17 mg) was cooled to -78 ^{0}C, pumped under high vacuum for 5 minutes, then nitrogen was let into the flask and the mixture was heated at 80 ^{0}C and stirred under nitrogen for 3 hours. The mixture was cooled to room temperature, diluted with ethyl acetate washed with saturated ammonium chloride, brine (3x), dried over magnesium sulfate, filtered and the solvent evaporated under vacuum. Purification by silica gel chromatography using 35% ethyl acetate/hexane as eluent afforded the title compound.

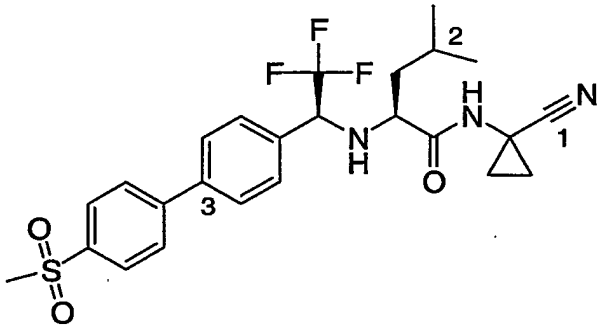
Step 3: 4-fluoro-N^1-[(2*R*,3*S*)-2-methyl-4-oxotetrahydrofuran-3-yl]-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide.

To a solution of the sulfide (120 mg) from Step 2 in ethyl acetate (2 mL) was added $Na_2WO_4 \bullet 2H_2O$ (1 mg) and n-Bu_4NHSO_4 (4 mg). Then 30 % hydrogen peroxide (0.06 mL) was slowly added and the mixture was stirred at room temperature for 2 hours. More hydrogen peroxide was then added (0.06 mL). Ethyl acetate was then added to the mixture which was washed with concentrated aqueous $Na_2S_2O_3$ (2x), brine, dried over magnesium sulfate, filtered and the solvent evaporated under vacuum. Purification by silica gel chromatography using 55% ethyl acetate/hexane as eluent afforded the title compound.

MS (+ESI): 559.1 $[M+1]^+$.

EXAMPLE 12

Synthesis of N^1-(1-cyano-1-methylethyl)-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide



Step 1: Preparation of 2-amino-2-methylpropanenitrile hydrochloride

To a 0 °C solution of ammonium chloride (15.5 g) in water (50 mL) was added a solution of acetone (17 mL) in diethyl ether (50 mL). Then a solution of the sodium cyanide (11.9 g.) in water (35 mL) was slowly added at such a rate that the temperature never exceeds 10 °C. The reaction mixture was stirred for one hour at 0 °C after the addition of the cyanide solution then it was allowed to stand overnight. The ether layer was separated and the aqueous layer was extracted with diethyl ether (2 x 30 mL). The combined organic layers were washed with brine, dried with magnesium sulfate and the solvent were removed in vacuo to yield a residue which was diluted with methyl alcohol (80 mL). The solution was cooled at –78 °C and saturated with ammonia gas (An Ace pressure tube with plunger valve and thermo well was used). The reaction mixture was allowed to stand at room temperature for two days.

The excess ammonia was expelled by a current of air and the methyl alcohol was removed by evaporation. The residue was dissolved in diethyl ether (50 mL) and cooled to 0 °C then a solution of 40 mL of hydrogen chloride (1.0 M in diethyl ether) was added. The mixture was stirred for 30 minutes and filtered to yield the title compound.

^{1}H NMR (CD_3SOCD_3) δ 9.45(1H, s), 1.70(6H, s).

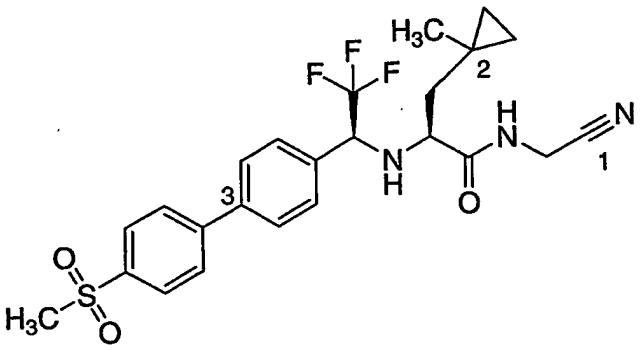
Step 2: Preparation of N^1-(1-cyano-1-methylethyl)-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide

To a DMF (30 mL) solution of the acid from Example 4, Step 6 (1.2 g) was added benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (1.55 g), 2-amino-2-methylpropanenitrile hydrochloride from Step 1 (720 mg) and the mixture was cooled to 0 °C. Triethylamine (1.3 mL) was added drop wise and the mixture was warmed to room temperature and stirred for 72 hours. It was poured into ice and saturated aqueous sodium bicarbonate and extracted with diethyl ether (3 X 50 mL). The combined extracts were washed with brine, dried with magnesium sulfate and the solvent removed in vacuo. The residue was purified by chromatography on SiO_2 using a gradient of ethyl acetate and hexanes (1:2 to 1:1) as eluant, followed by trituration using diethyl ether and hexanes to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 8.08(2H, d), 7.95(2H, d), 7.80(2H, d), 7.68(1H, bs), 7.65(2H, d), 4.32-4.42 (1H, m), 3.43-3.52 (1H, m), 3.20 (3H, s), 2.65-2.75(1H, m), 1.90-2.00(1H, m), 1.57(3H, s), 1.52(3H, s), 1.52-1.57 (1H, m), 1.40-1.50(1H, m), 0.90-0.98(6H, m).

EXAMPLE 13

N^1-(Cyanomethyl)-3-(1-methylcyclopropyl)-N^2-{2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-alaninamide



Step 1: Methyl *N*-(diphenylmethylene)-4-methylenenorvalinate

To a solution of methyl *N*-(diphenylmethylene)glycinate (12.0 g, 47.4 mmol) in THF (118 mL) at 0 °C was added a solution of 1M potassium *tert*-butoxide in THF (49 mL, 49 mmol). The mixture turned bright yellow and was further stirred for ~ 15 min. 3-Bromo-2-methylpropene (5.2 mL, 51.3 mmol) was added and the mixture was stirred at room temperature for 2 days. After quenching with water, the mixture was extracted with EtOAc. Chromatography over silica gel and elution with hexanes:EtOAc (6:1) provided 2.2 g of dialkylated product as the less polar component. Further elution gave the title compound as the more polar component.

^{1}H NMR (Acetone-d_6) δ 7.62 – 7.18 (m, 10H), 4.72 (s, 1H), 4.64 (s, 1H), 4.20 (dd, 1H), 3.64 (s, 3H), 2.62 (dd, 1H), 2.50 (dd, 1H), 1.48 (s, 3H).

Step 2: Methyl *N*-[(benzyloxy)carbonyl]-4-methylenenorvalinate

A mixture of methyl *N*-(diphenylmethylene)-4-methylenenorvalinate (6.2 g, 20.2 mmol) from Step 1 and 0.5 M of aqueous HCl (60 mL) was stirred at room temperature overnight. The whole mixture was washed with Et_2O (2x). After cooling to 0 °C, 1M aqueous NaOH (40 mL, 40 mmol) was added, followed by EtOAc (50 mL) and benzyl chloroformate (4 mL, 28 mmol). The mixture was stirred at 0 °C for 2h. The EtOAc layer was then separated, washed with water (2x), dried ($MgSO_4$) and concentrated. Chromatography over silica gel and elution with hexanes:EtOAc (6:1), then (3:1) gave the title compound as a colorless oil.

^{1}H NMR (Acetone-d_6) δ 7.40 – 7.25 (m, 5H), 6.54 (d, 1H), 5.06 (s, 2H), 4.82 (s, 1H), 4.78 (s, 1H), 4.40 (m, 1H), 3.68 (s, 3H), 2.52 (dd, 1H), 2.40 (dd, 1H), 1.74 (s, 3H).

Step 3: Methyl *N*-[(benzyloxy)carbonyl]-3-(1-methylcyclopropyl)alaninate

To CH_2Cl_2 (40 mL) at 0 °C was added diethylzinc (2 mL, 19.5 mmol), followed by dropwise addition of a solution of trifluoroacetic acid (1.5 mL, 19.5 mmol) in CH_2Cl_2 (8 mL). After stirring for 15 min, a solution of diiodomethane (1.6 mL, 20.0 mmol) in CH_2Cl_2 (8 mL) was added. The mixture was stirred for 15 min and a clear solution resulted. A solution of methyl *N*-[(benzyloxy)carbonyl]-4-methylenenorvalinate (2.75 g, 9.9 mmol) from step 2 was added and the mixture was stirred at room temperature overnight. After quenching with 0.1 M aqueous HCl (50 mL), the CH_2Cl_2 layer was separated, washed with diluted brine, dried ($MgSO_4$) and concentrated to give the crude tilte compound.

^{1}H NMR (Acetone-d_6) δ 7.35 (m, 5H), 6.58 (d, 1H), 5.08 (m, 2H), 4.40 (m, 1H), 3.68 (s, 3H), 1.75 (dd, 1H), 1.62 (dd, 1H), 1.08 (s, 3H), 0.45 (m, 1H), 0.22 (m, 3H).

Step 4: Benzyl 2-hydroxy-1-[(1-methylcyclopropyl)methyl]ethylcarbamate

To a solution of the crude ester from step 3 in EtOH (40 mL) and THF (40 mL) and cooled at 0 °C was added LiCl (1.7g), followed by $NaBH_4$ (1.6 g). The mixture was stirred at room temperature overnight, quenched with 0.5 M aqueous HCl, extracted with EtOAc. The EtOAc extract was washed with diluted brine (2x), dried ($MgSO_4$) and concentrated. Purification by chromatography gave 2 g of the title compound as a colorless oil.

^{1}H NMR (Acetone-d_6) δ 7.35 (m, 5H), 5.98 (d, 1H), 5.06 (m, 2H), 3.85 (m, 1H), 3.72 (t, 1H), 3.50 (m, 2H), 1.60 (dd, 1H), 1.34 (dd, 1H), 1.05 (s, 3H), 0.40 – 0.15 (m, 4H).

Step 5: 2-Amino-3-(1-methylcyclopropyl)propan-1-ol

A mixture of benzyl 2-hydroxy-1-[(1-methylcyclopropyl)methyl]-ethylcarbamate (2.0 g, 7.6 mmol) and 10% Pd/C (200 mg) in EtOH (80 mL) with 2 mL of 1 M aqueous HCl was stirred H_2 atmosphere (balloon) overnight. The catalyst was filtered off and the filtrate was concentrated to give the title compound.

^{1}H NMR (Methanol-d_4) δ 3.72 (dd, 1H), 3.40 (dd, 1H), 3.22 (m, 1H), 1.50 – 1.30 (m, 2H), 1.06 (s, 3H), 0.45 – 0.25 (m, 4H).

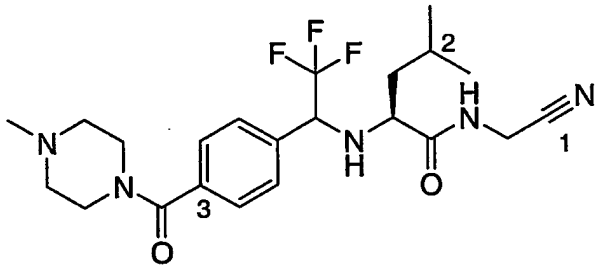
Step 6: N^1-(Cyanomethyl)-3-(1-methylcyclopropyl)-N^2-{2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-alaninamide

The title compound was prepared from the aminoalcohol from Step 5 using the same method described in Example 5, Steps 6-12.

^{1}H NMR (Acetone-d_6) δ 8.06 (br s, 1H), 8.00 (d, 2H), 7.94 (d, 2H), 7.78 (d, 2H), 7.62 (d, 2H), 4.48 (m, 1H), 4.12 (m, 2H), 3.55 (m, 1H), 3.16 (s, 3H), 1.64 (m, 2H), 1.10 (s, 3H), 0.48 – 0.20 (m, 4H).
MS (+ESI): 494 (MH^+).

EXAMPLE 14

Synthesis of N^1-(cyanomethyl)-N^2-(2,2,2-trifluoro-1-{4-[(4-methylpiperazin-1-yl)carbonyl]phenyl}ethyl)-L-leucinamide



Step 1: N^2-{1-[4-(hydroxycarbonyl)phenyl]-2,2,2-trifluoroethyl}-N^1-(cyanomethyl)-L-leucinamide

A mixture of dichlorobis(triphenylphosphine)palladium(II) (58 mg, 0.08 mmol), triphenylphosphine (155 mg, 0.59 mmol) and N^2-[1-(4-bromophenyl)-2,2,2-trifluoroethyl]-N^1-(cyanomethyl)-L-leucinamide (Example 2, 1.2 g, 3.0 mmol) in tributylamine (2.5 mL) and water (0.6 mL) was placed in a steel bomb with a teflon coated magnetic bar. The system was purged 3 times with carbon monoxide (100 psi each times) and finally filled with this gas at a pressure of 300 psi. The reaction mixture was heated with continuous stirring at 160°C for 20 hours. Then the system was allowed to cool to room temperature, pressure was released and the resulting residue was partitioned between EtOAc and water + aqueous hydrochloric acid to adjust the pH between 2.5 and 3.0. The organic layer was dried over Na2SO4, filtered and concentrated. The crude product was purified by chromatography using EtOAc, hexane and acetic acid as eluant to give the title compound as a yellow-orange foam.

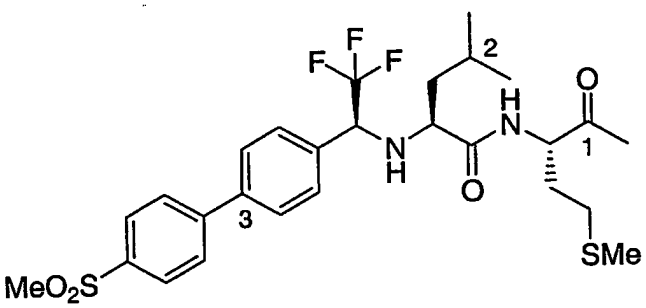
Step 2: N^1-(cyanomethyl)-N^2-(2,2,2-trifluoro-1-{4-[(4-methylpiperazin-1-yl)carbonyl]phenyl}ethyl)-L-leucinamide

To a solution of N^2-{1-[4-(hydroxycarbonyl)phenyl]-2,2,2-trifluoroethyl}-N^1-(cyanomethyl)-L-leucinamide from step 1 (480 mg, 1.3 mmol) and benzotriazol-1-yloxytrpyrrolidinophosphonium hexafluorophosphonate (1.35 g, 2.6 mmol) in DMF (8 mL) was slowly added *N*-methylpiperazine (0.29 mL, 2.6 mmol) followed by triethylamine (0.54 mL, 3.9 mmol). The reaction mixture was stirred at room temperature for 3 hours. The resulting mixture was partitioned between EtOAc and water + aqueous hydrochloric acid to adjust the pH between 2.5 and 3.0. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography using MeOH, EtOAc and NH_4OH_{conc} as eluant to give the title compound as a white foam.

MS (+ESI): 454.3 $[M+1]^+$.

EXAMPLE 15

Synthesis of N^1-{(1S)-1-[2-(methylthio)ethyl]-2-oxopropyl}-N^2-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide



Step 1: N^2-(tert-Butoxycarbonyl)-N^1-methoxy- N^1-methyl-L-methioninamide

To an ice-cold solution of *N*-Boc-L-methionine (1.0 g, 4.0 mmol), *O*-(7-azabenzotriazol-1-yl)-*N*, *N*, *N'*, *N'*-tetramethyluronium hexafluorophosphate (3.3 g, 8.7 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (1.0 g, 10.2 mmol) in DMF (15 mL) was added triethylamine (2.2 mL, 15.8 mmol) dropwise. The resulting mixture was stirred at room temperature for 18 hours and then partitioned between EtOAc and half-saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography using EtOAc and hexane as eluant to give the title compound as a colorless syrup.

Step 2: *tert*-Butyl {(1S)-1-[2-(methylthio)ethyl]-2-oxopropyl}carbamate

To a solution of N^2-(tert-butoxycarbonyl)-N^1-methoxy- N^1-methyl-L-methioninamide from Step 1 (200 mg, 0.68 mmol) in THF cooled to -78°C was added a solution of methyl lithium 1.4 M in hexane (1.1 mL, 1.5 mmol). The reaction was stirred at this temperature for 2 hours and then cold-quenched with an aqueous solution of ammonium acetate 25%$^{W}/_{V}$. The mixture was extracted with EtOAc, the organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography using EtOAc and hexane as eluant to give the title compound as a colorless gum.

Step 3: (3S)-3-Amino-5-(methylthio)pentan-2-one, hydrochloride

To *tert*-butyl {(1S)-1-[2-(methylthio)ethyl]-2-oxopropyl}carbamate from step 2 (140 mg, 0.57 mmol) was added a solution hydrogen chloride 4.0 M in 1,4-dioxane (3 mL, 12 mmol). The resulting mixture was stirred at room temperature for 1 hour. The solvent was removed in vacuo and the resulting residue azeotroped with toluene (2 X 10 mL) to give the title compound as a white solid.

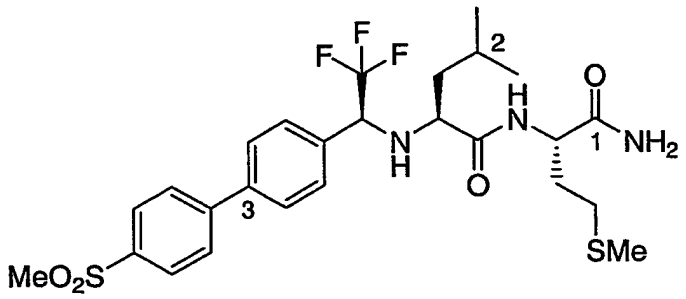
Step 4: N^1-{(1S)-1-[2-(methylthio)ethyl]-2-oxopropyl}-N^2-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide

A suspension of (3S)-3-amino-5-(methylthio)pentan-2-one, hydrochloride from Step 3 (104 mg, 0.57 mmol), *N*-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucine (Example 4, Step 6, 127 mg, 0.2 mmol) and *O*-(7-azabenzotriazol-1-yl)-*N*, *N*, *N'*, *N'*-tetramethyluronium hexafluorophosphate (187 mg, 0.49 mmol) in DMF (1 mL) was cooled to 10°C and then triethylamine (115 µL, 0.83 mmol) was added slowly . The reaction mixture was stirred at room temperature for 3 hours. The resulting mixture was partitioned between EtOAc and half-saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography using EtOAc and hexane as eluant to give the title compound as a white foam.

MS (+ESI): 573.5 $[M+1]^+$.

EXAMPLE 16

Synthesis of N^1-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl-L-methioninamide

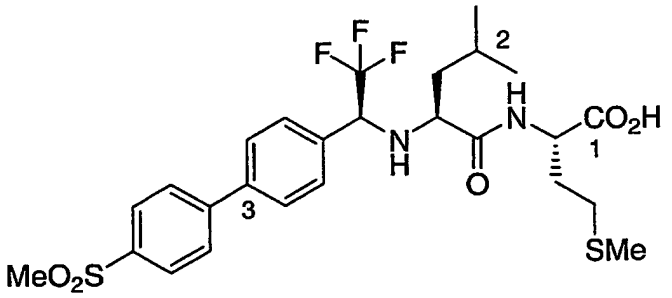


Using the procedure described for Example 15, Step 4, where (3S)-3-amino-5-(methylthio)pentan-2-one, hydrochloride was substituted for L-methioninamide hydrochloride, the title compound was obtained and was crystallized from EtOAc and hexane (1:2) to give a white solid.

MS (+ESI): 574.3 $[M+1]^+$.

EXAMPLE 17

Synthesis of *N*-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl-L-methionine



Step 1: Methyl *N*-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl-L-methioninate

Using the procedure described for Example 15, Step 4, where (3S)-3-amino-5-(methylthio)pentan-2-one, hydrochloride was substituted for methyl L-methionate hydrochloride, the title compound was obtained and was crystallized from EtOAc and hexane (1:2) to give a white solid.

MS (+ESI): 589.3 $[M+1]^+$.

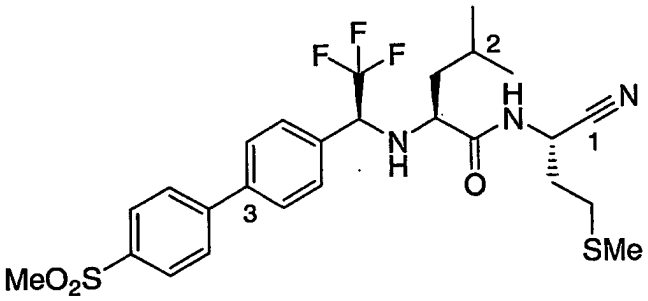
Step 2: *N*-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl-L-methionine

To an ice-cold solution of methyl *N*-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl-L-methioninate from Step 1 (100 mg, 0.17 mmol) in THF (2 mL) and MeOH (0.5 mL) was added 1.0N LiOH dropwise (0.25 mL, 0.25 mmol) and the resulting solution was stirred at room temperature for 18 hours. The solution was partitioned between EtOAc and water + 1N HCl (1 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography using EtOH, EtOAc and AcOH as eluant to give the title compound as a white foam which was crystallized from EtOAc and hexane (6 mL, 1:2) to give a white solid.

MS (+ESI): 575.0 $[M+1]^+$.

EXAMPLE 18

Synthesis of N^1-[(1S)-1-cyano-3-(methylthio)propyl]-N^2-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide

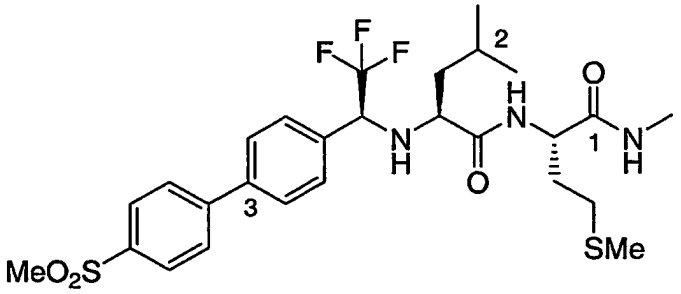


A solution of N^1-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl-L-methioninamide from Example 16 (350 mg, 0.6 mmol) and pyridine (85 μL, 1.05 mmol) in 1,4-dioxane was cooled to 10 °C. Then, trifluoroacetic anhydride was added dropwise (65 μL, 0.46 mmol) and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was partitioned between EtOAc and water. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography using EtOAc and hexane as eluant to give the title compound as a colorless gum.

MS (+ESI): 556.3 $[M+1]^+$.

EXAMPLE 19

Synthesis of *N*-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl-N^1-methyl-L-methioninamide

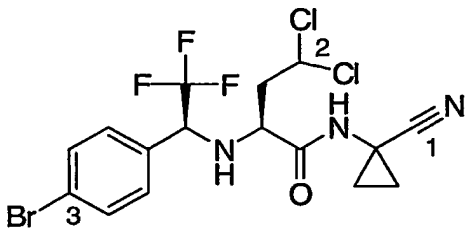


A suspension of *N*-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl-L-methionine from Example 17 (100 mg, 0.17 mmol), methylamine hydrochloride (35 mg, 0.52 mmol) and *O*-(7-azabenzotriazol-1-yl)-*N*, *N*, *N'*, *N'*-tetramethyluronium hexafluorophosphate (150 mg, 0.39 mmol) in DMF (1 mL) was cooled to 10°C and then triethylamine (110 μL, 0.79 mmol) was added slowly . The reaction mixture was stirred at room temperature for 18 hours. The resulting mixture was partitioned between EtOAc and half-saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography using EtOAc and hexane as eluant to give the title compound as a white foam which was crystallized from EtOAc and hexane to give a white solid.

MS (+ESI): 588.1 $[M+1]^+$.

EXAMPLE 20

Synthesis of (2S)-2-{[(1S)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]amino}-4,4-dichloro-N-(1-cyanocyclopropyl)butanamide



Step 1 Ethyl (2S)-2-amino-4,4-dichlorobutanoate

To ice cold ethanol, 25 mL was added dropwise acetyl chloride (2.0 mL, 28 mmol). (S)-2-Amino-4,4-dichlorobutanoic acid (1.0 g, 5.8 mmol) [prepared according to *Chem. – Ztg* 114, 249-251 (1990) and *Synthesis* 1996, 1419] was then added in one portion. The mixture was refluxed for 18 h, concentrated and the residue partitioned between saturated $NaHCO_3$ solution and dichloromethane. The organics were separated dried (Na_2SO_4), filtered and concentrated to give an oily residue which was purified through a silica gel plug, eluting with 30% ethyl acetate in hexanes to give pure title compound.

Step 2 Ethyl(2S)-2-{[(1S)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]amino}-4,4-dichlorobutanoate

Ethyl (2S) –2-amino-4,4-dichlorobutanoate (298 mg, 1.49 mmol), (1S)-1-(4-bromophenyl)-2,2,2-trifluoroethyl trifluoroacetate (862 mg, 2.2 mmol) [prepared according to *J. Am. Chem. Soc.* 1983, 105, 2343-2350] and diisopropylethylamine (284 mg, 2.2 mmol) were heated neat at 60°C and under N_2 atmosphere for 6 h. The mixture was partitioned between $NaHCO_3$ solution and ethyl acetate. The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. Purification by ISCO column chromatography (gradient 2% to 15% ethyl acetate/hexanes) provided the title compound as a 87:13 mixture of diastereomers.

MS (+APCI): 438.8 [M+1] and 440.8 [M+3].

Step 3 (2S)-2-{[(1S)-1-(4-Bromophenyl)-2,2,2-trifluoroethyl]amino}-4,4-dichlorobutanoic acid

To a solution of ethyl (2S)-2-{[(1S)-1-(4-bromophenyl)-2,2,2-trifluoroethyl]amino}-4,4-dichlorobutanoate (325 mg, 0.74 mmol) in THF (5 mL) was added potassium trimethylsilanolate (178 mg, 1.39 mmol). The mixture was stirred for 1.5 h at room temperature and then concentrated. The

residue was partitioned between ethyl acetate and 1N HCl. The organic layer was separated, dried (Na_2SO_4), filtered and concentrated to obtain the title compound as an oil which was used as such in the next step.

MS (-APCI): 407.9 [M-1].

Step 4 (2S)-2-{[(1S)-1-(4-Bromophenyl)-2,2,2-trifluoroethyl]amino}-4,4-dichloro-N-(1-cyanocyclopropyl)butanamide

A mixture of (2S)-2-{[(1S)-1-(4-bromophenyl)-2,2,2-trifluoroethyl] amino}-4,4-dichlorobutanoic acid (275 mg, 0.67 mmol), 1-amino-1-cyclopropane carbonitrile hydrochloride (159 mg, 1.34 mmol) and HATU coupling reagent (305 mg, 0.8 mmol) was dissolved in DMF (4 mL). Triethylamine (0.3 mL, 2.1 mmol) was added and the mixture stirred overnight and then poured into $NaHCO_3$ solution and ethyl acetate. The organic layer was separated, washed with brine, 1N HCl and brine again. The organic layer was separated, dried (Na_2SO_4), filtered and concentrated to give 393 mg of an oil which was purified by column chromatography eluting with 6:3:1 toluene: ethyl acetate: dichloromethane. Swishing the pure product with diethyl ether gave the title compound as a white solid and as a 85:15 mixture of diastereomers.

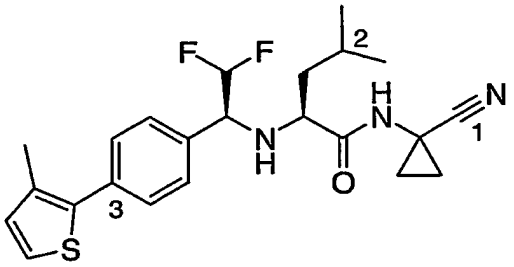
MS (-APCI): 471.9 [M-1].

^{1}H NMR (500 MHz, DMSO-d_6) "major isomer", δ 8.95 (s, 1H), 7.61 (d, 2H, 7.38 (d, 2H), 6.19 (dd, 1H), 4.38 – 4.25 (m, 1H), 3.45 - 3.38 (bs, 2H), 2.5 – 2.3 (m, 2H), 1.43 -1.32 (m, 2H), 1.02 - 0.95 (m, 1H), 0.75 - 0.68 (m, 1H).

^{1}H NMR (500 MHz, DMSO-d_6) "minor isomer", δ 8.89 (s, 1H), 7.65 – 7.61 (m, 1H), 7.43 (d, 1H), 6.23 – 6.19 (m, 1H), 4.38 – 4.25 (m, 1H), 3.45 - 3.38 (bs, 2H), 2.5 - 2.3 (m, 2H), 1.43 - 1.32 (m, 2H), 1.02 - 0.95 (m, 1H), 0.75 - 0.68 (m, 1H).

EXAMPLE 21

N^1-(1-cyanocyclopropyl)-N^2-{(1*S*)-2,2-difluoro-1-[4-(3-methyl-2-thienyl)phenyl]ethyl}-L-leucinamide.



Step 1: Preparation of (2*S*)-1-{[tert-butyl(dimethyl)silyl]oxy}-*N*-[(1*Z*)-2,2-difluoroethylidene]-4-methylpentan-2-amine

A mixture of (2*S*)-1-{[tert-butyl(dimethyl)silyl]oxy}-4-methylpentan-2-amine (Example 4, Step 1, 8.5 g, 36.8 mmol) and difluoroacetaldehyde ethyl hemiacetal (5.0g, 39.7 mmol) in benzene was refluxed with a Dean-stark trap overnight. Solvent was removed in vacuo. The residue was passed through a short silica column and eluted with hexanes: EtOAc (10:1) to give the title compound as a pale yellow oil.

^{1}H NMR (CD_3COCD_3) δ 7.72(m, 1H), 6.12(dt, 1H), 3.70(dd, 1H), 3.54(dd, 1H), 3.36(m, 1H), 1.48(m, 2H), 1.32(m, 1H), 0.95 – 0.78(m, 15H), 0.06(s, 3H), 0.02(s, 3H).

Step 2: Preparation of (2*S*)-2-{[(1*S*)-1-(4-bromophenyl)-2,2-difluoroethyl]amino}-4-methylpentan-1-ol

n-BuLi (2.5 M in hexanes, 1.43 mL) was added to a –70 °C THF (8.5 mL) solution of 1,4-dibromobenzene (884 mg) and the mixture was stirred for 15 minutes. A THF (8.5 mL) solution of (2*S*)-1-{[*tert*-butyl(dimethyl)silyl]oxy}-4-methyl-*N*-[(1*E*)-2,2-difluoroethylidene]pentan-2-amine (1.0 g) was then added dropwise and the mixture was stirred for 1.5 hours. It was then poured slowly into an icy saturated solution of ammonium chloride under vigorous stirring. It was extracted with 3 portions of ethyl acetate. The combined organic layers were washed with brine, dried with magnesium sulfate and the solvent was removed in vacuo to yield a residue, which was purified on SiO_2 using a gradient of hexanes and ethyl acetate (90:10 to 75:25) as eluent to yield the title compound. The compound (200 mg) from above was dissolved in CH_3CN (4 mL) and the solution was cooled to 0 °C. HF-pyridine (40 □M) was added dropwise and the mixture was reacted for 16 hours. It was poured into a saturated solution of sodium bicarbonate, ethyl acetate was added and it was vigorously shaken. The organic layer was separated and the aqueous further extracted with ethyl acetate (2 X 50 mL). The combined organic

layers were washed with brine, dried with magnesium sulfate and the solvent was removed in vacuo to yield a residue which was purified on SiO_2 using a gradient of hexanes and ethyl acetate (80:20 to 60:40) as eluent to yield the title compound.

^{1}H NMR (CD_3COCD_3) δ 7.6(2H, d), 7.45(2H, d), 6.0 (1H, dt), 4.25(1H, m), 3.65 (1H, t), 3.5-3.55(1H, m), 3.3-3.35(1H, m), 2.55-2.65(1H, m), 2.15-2.25(1H, m), 1.6-1.7(1H, m), 1.3-1.4(1H, m), 1.2-1.3(1H, m), 0.9(3H, d), 0.8(3H, d).

Step 3: Preparation of *N*-[(1*S*)-1-(4-bromophenyl)-2,2-difluoroethyl]-L-leucine

A suspension of H_5IO_6 /CrO_3 (5.5 mL of 0.40 M in CH3CN; see Note below) was cooled to 0 °C and a solution of the alcohol from Step 2 (250 mg) in CH_3CN (3.7 mL) was added dropwise. The mixture was stirred at 0-5 °C for 3.5 hours. After this period, 2.0 mL of the oxidant were added. After 1.5 hours it was poured into Na_2HPO_4 buffer (0.4g in 10 mL) under vigorous stirring and the mixture was extracted with diethyl ether (3 X 20 mL). The combined ether extracts were washed with water and brine (1:1), with dilute aqueous $NaHSO_3$ and brine. The organic extract was dried with magnesium sulfate, filtered and the solvent was evaporated to dryness to yield a residue that was used without further purification.

Note: The oxidizing reagent (H_5IO_6 /CrO_3) was prepared as described in *Tetrahedron Letters* 39 (1998) 5323-5326 but using HPLC grade CH_3CN (contains 0.5% water); no water was added.

^{1}H NMR (CD_3COCD_3) δ 7.55(2H, d), 7.4(2H, d), 6.05(1H, dt), 3.95-4.05(1H, m), 3.45(1H, t), 2.7-3.0(broad m, NH/OH), 1.85-1.95(1H, m), 1.5(2H, t), 0.95 (3H, d), 0.9(3H, d).

Step 4: Preparation of N^2-[(1*S*)-1-(4-bromophenyl)-2,2-difluoroethyl]-N^1-(1-cyanocyclopropyl)-L-leucinamide

To a DMF (2 mL) solution of the acid from Step 3 (258 mg) were added O-(7-azabenzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate (337 mg), 1-aminocyclopropanecarbonitrile hydrochloride (175 mg). After 1 minute of stirring, diisopropylethylamine (0.45 mL) was added dropwise and the mixture was stirred for 16 hours. It was poured into saturated aqueous sodium bicarbonate and extracted with ethyl acetate (3 X 15 mL). The combined extracts were washed with brine, dried with magnesium sulfate and the solvent removed in vacuo. The residue was purified by chromatography on SiO_2 using hexanes and ethyl acetate (80:20 to 50:50).

^{1}H NMR (CD_3COCD_3) δ 8.05(1H, m), 7.55(2H, d), 7.4(2H, d), 6.05(1H, dt), 3.95-4.05(1H, m), 3.25-3.3(1H, m), 2.4-2.45(1H, m), 1.8-1.9 (1H, m), 1.4-1.55(2H, m), 0.95-1.1 (2H, m), 0.95(6H, t).

Step 5: Preparation of *N*1-(1-cyanocyclopropyl)-*N*2-{(1*S*)-2,2-difluoro-1-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]ethyl}-L-leucinamide.

To a DMF (60 mL) solution of the arylbromide from Step 4 (5.23 g) and of bis(pinacolato)diboron (3.8 g) were added potassium acetate (3.7 g) and $PdCl_2$dppf (309 mg). A stream of nitrogen was passed through the suspension for 1 minute. The reaction mixture was heated at 80°C for 16h. It was allowed to cool to room temperature and transferred to a sep. funnel. A saturated solution of $NaHCO_3$ (~120 mL) and EtOAc (100 mL) were added. Organic layer was separated and the aqueous layer was further extracted with 2 portions of EtOAc (2X100mL). Combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. Crude material was purified on silica gel (80:20 to 50:50 hex/EtOAc) to yield the desired boronate.

^{1}H NMR (CD_3COCD_3) δ 8.15(bs, NH), 7.72(2H, d), 7.40(2H, d), 6.02(1H, dt), 3.95(1H, m), 3.25(1H, q), 2.38(1H, m), 1.72(1H, m), 1.27-1.50(16H, m), 0.85-1.05(8H, m).

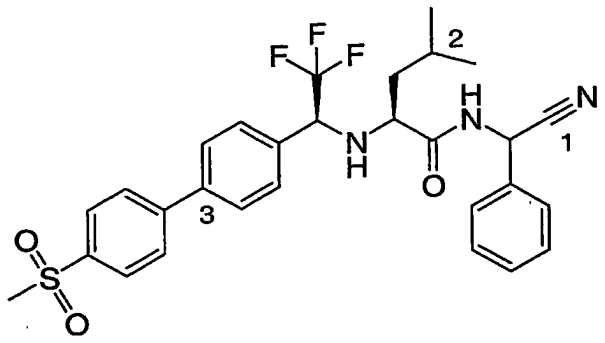
Step 6: Preparation of N^1-(1-cyanocyclopropyl)-N^2-{(1*S*)-2,2-difluoro-1-[4-(3-methyl-2-thienyl)phenyl]ethyl}-L-leucinamide.

In a sealable tube for microwave, a stream of nitrogen was passed through a suspension made of the aryl boronate from Step 6 (200 mg), 2-bromo-3-methylthiphene (115 mg), 2 M Na_2CO_3 (0.65 mL), DMF (4.3 mL) and $PdCl_2$dppf (11 mg) for 1 minute. The mixture was then heated in microwave (SmithCreator) for 500 seconds (fixed hold time: OFF) at 120°C (absorption level: high). It was cooled to room temperature, diluted with ethyl acetate (20 mL) and poured into a saturated solution of sodium bicarbonate. The ethyl acetate layer was separated and the aqueous further extracted with ethyl acetate (2 X 15 mL). The combined ethyl acetate extracts were washed with brine and dried with magnesium sulfate. Removal of the solvent left a residue that was purified by chromatography on SiO_2 using a gradient of hexanes and ethyl acetate (80:20 to 50:50 hex/EtOAc).

^{1}H NMR (CD_3COCD_3) δ 8.13(bs, NH), 7.50(4H, s), 7.37(1H, d), 6.97(1H, d), 6.05(1H, t), 4.00(1H, m), 3.30(1H, m), 2.42(1H, m), 2.32(3H, s), 1.85(1H, m), 1.40-1.53(2H, m), 1.30-1.40(2H, m), 0.85-1.03(8H, m).

EXAMPLE 22

Synthesis of N^1(1-cyanotolyl)-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucinamide

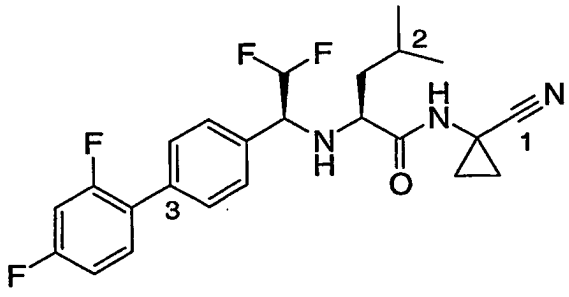


To a mixture of N-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucine (Example 4, Step 6, 21 mg, 0.046 mmol) in DMF (1.4 mL) is added 2-phenylglycinonitrile hydrochloride (8.6 mg, 0.051 mmol). N-methylmorpholine (41 μL, 0.368 mmol) and N-propylphosphonic acid anhydride, cyclic trimer 50% (55 μL, 0.092 mmol) were added sequentially and the mixture was stirred overnight. The volatiles were evaporated in a Genevac HT-4. The residue was dissolved in CH_2Cl_2 (5 mL) treated 30 min with BTMA carbonate silica gel and filtered through a SiOH SPE cartridge (500 mg). The solution was treated with Amberlyst A-21 and filtered again. The solution was concentrated to give a 1:1 mixture of epimers.

MS (-ESI): 556.0 $[M-1]^-$

EXAMPLE 23

Synthesis of N^1(cyanocyclopropyl)-N^2-{(1S)-2,2-difluoro-1-[2',4'-difluoro-1,1'-biphenyl-4-yl]ethyl}-L-leucinamide

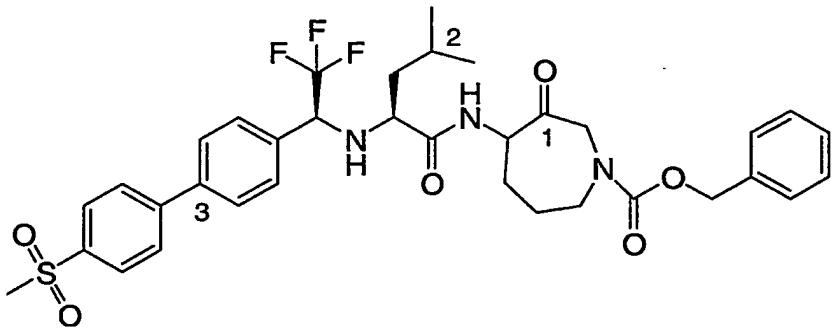


Using the procedure described for Example 21, where 2-bromo-3-methylthiophene in Step 6 was replaced by 1-bromo-2,4-difluorobenzene, the title compound was obtained as a white solid.

MS (+API): 448.1 $[M+1]^+$.

EXAMPLE 24

Synthesis of benzyl 3-oxo-4-[(*N*-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl)amino]azepane-1-carboxylate



Step 1: Benzyl 3-hydroxy-4-[(*N*-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl)amino]azepane-1-carboxylate

To a 0 °C solution of N-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)-1,1'-biphenyl-4-yl]ethyl}-L-leucine (Example 4, Step 6, 605 mg, 1.37 mmol), benzyl 4-amino-3-hydroxyazepane-1-carboxylate (WO 0134565, *J. Med. Chem.* ***44***, 1380, **2001**, 326 mg, 1.23 mmol), and PyBOP (724 mg, 1.39 mmol) in 10 mL of DMF was added triethylamine (0.45 mL, 3.2 mmol). The mixture was stirred 1h, warmed to room temperature for 1h, then partitioned between $NaHCO_3$ and ether. The organic phase was washed with pH 3.5 phosphate buffer, then brine, and dried over $MgSO_4$. Purification by silica gel chromatography (gradient 65% ethyl acetate:hexanes to 100% ethyl acetate) provided the title compound as a mixture of isomers.

Step 2: Benzyl 3-oxo-4-[(*N*-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl)amino]azepane-1-carboxylate

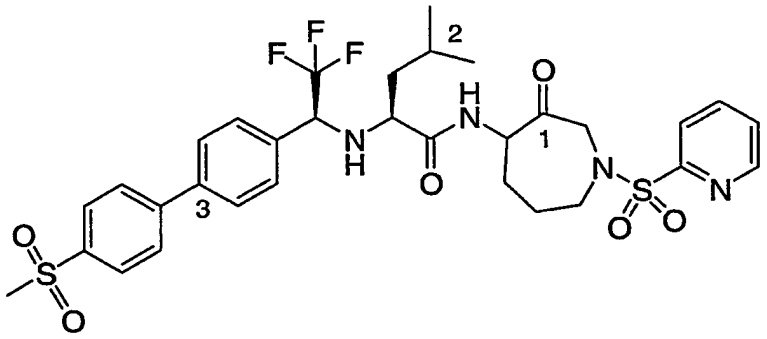
To a 0 °C solution of benzyl 3-hydroxy-4-[(*N*-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl)amino]azepane-1-carboxylate (98 mg, 0.14 mmol) in dichloromethane (3 mL) was added Dess-Martin periodinane. The mixture was warmed to room temperature, stirred 15h, then partitioned between ethyl acetate and 1M NaOH. The organic phase was

washed with brine and dried over $MgSO_4$. Purification by silica gel chromatography (gradient 40% to 70% ethyl acetate:hexanes) provided the title compound as a mixture of diastereomers.

MS (+ESI): 688.4 $[M+1]^+$

EXAMPLE 25

Synthesis of N^1-[3-oxo-1-(pyridin-2-ylsulfonyl)azepan-4-yl]-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide



Step 1: N^1-(3-hydroxyazepan-4-yl)-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide

A mixture of benzyl 3-hydroxy-4-[(*N*-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucyl)amino]azepane-1-carboxylate (Example 24, Step 1, 710 mg, 1.03 mmol) and 10% Pd/C (490 mg) in 2:1 EtOH:EtOAc (80 mL) was flushed with hydrogen and stirred under a hydrogen balloon for 2h. The reaction mixture was filtered through celite and concentrated to give the title compound.

Step 2: N^1-[3-hydroxy-1-(pyridin-2-ylsulfonyl)azepan-4-yl]-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide

To a 0 °C solution of N^1-(3-hydroxyazepan-4-yl)-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide (567 mg, 1.02 mmol) in dichloromethane (10 mL) was added triethylamine (0.25 mL, 1.8 mmol) and 2-pyridinesulfonyl chloride (204 mg, 1.15 mmol). The mixture was warmed to room temperature for 1h, then partitioned between dichloromethane and $NaHCO_3$. The organic phase was washed with brine, filtered through cotton and concentrated. Purification by silica gel chromatography (gradient 70% ethyl acetate:hexanes to 100% ethyl acetate) provided the title compound as a mixture of isomers.

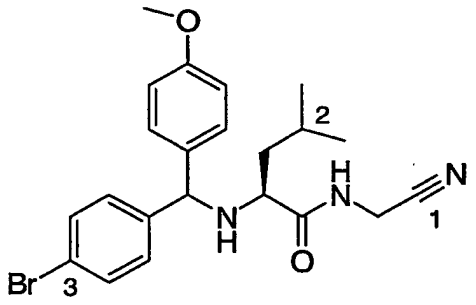
Step 3: N^1-[3-oxo-1-(pyridin-2-ylsulfonyl)azepan-4-yl]-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide

To a room temperature solution of N^1-[3-hydroxy-1-(pyridin-2-ylsulfonyl)azepan-4-yl]-N^2-{(1*S*)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide (460 mg, 0.73 mmol) in dichloromethane (15 mL) was added Dess-Martin periodinane (420 mg, 1.0 mmol). The mixture was stirred 1h, then diluted with dichloromethane, washed with 1M NaOH, then brine. The organic phase was filtered through cotton and concentrated. Purification by silica gel chromatography (gradient 60% ethyl acetate:hexanes to 100% ethyl acetate) provided the title compound as a mixture of diastereomers.

MS (+ESI): 695.3 $[M+1]^+$

EXAMPLE 26

Synthesis of N^2-[(4-bromophenyl)(4-methoxyphenyl)methyl]-N^1-(cyanomethyl)-L-leucinamide



Step 1: (4-bromophenyl)(4-methoxyphenyl)methanol

A solution of 1,4-dibromobenzene (9.1 g, 38 mmoles) in THF (80 mL) was cooled to –78 °C and n-butyllithium (16 mL, 2.5M in hexanes) was added dropwise. After 15 minutes, p-anisaldehyde (5 g, 37 mmoles in 4.5 mL of THF) was added dropwise. After another 20 minutes, the reaction mixture was quenched with methanol (5 mL) and saturated aqueous ammonium chloride (100 mL). The reaction mixture was extracted with three 100-mL portions of ethyl acetate and the combined organic layers were washed with brine (50 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to give the title compound which was used directly in the next step.

Step 2: Methyl *N*-[(4-bromophenyl)(4-methoxyphenyl)methyl]-L-leucinate

(4-Bromophenyl)(4-methoxyphenyl)methanol (1.15 g, 3.9 mmoles) was dissolved in dichloromethane (4 mL) along with tetrabutylammonium bromide (130 mg, 0.4 mmoles). 48% aqueous hydrobromic acid (3.3 mL) was then added and the reaction mixture stirred vigourously for 40h. The products were partitioned between water (20 mL) and dichloromethane (30 mL) and dried over magnesium sulfate. The organic layer was concentrated to approximately 10 mL and L-leucine methyl ester (free base) was added as a solution in dichloromethane (20 mL) followed by triethylamine (3 mL). The reaction mixture was stirred at 35 °C for 20 minutes (a white precipitate appeared). The reaction was taken-up in ether (50 mL) and water (30 mL). The phases were separated and the organic layer washed with saturated aqueous ammonium chloride solution (30 mL) and brine (30 mL). After drying over magnesium sulfate and concentrating under reduced pressure, methyl *N*-[(4-bromophenyl)(4-methoxyphenyl)methyl]-L-leucinate was obtained and purified on silica gel using 10% ethyl acetate, 90% hexanes.

Step 3: *N*-[(4-bromophenyl)(4-methoxyphenyl)methyl]-L-leucine

To a room temperature solution of methyl *N*-[(4-bromophenyl)(4-methoxyphenyl)methyl]-L-leucinate (1.25g, 3 mmol) in 60 mL of approximately 2:1:1 THF/MeOH/Water was added lihium hydroxide monohydrate (250 mg, 6 mmoles). The mixture was stirred overnight and concentrated. The residue was partitioned between dichloromethane (50 mL) and pH 3.5 phosphate buffer (50 mL). The aqueous phase was separated and washed with two 50-mL portions of dichloromethane. The organic phase was dried over sodium sulfate and concentrated under reduced pressure to yield a solid that was triturated in a minimun of cold dichloromethane to give *N*-[(4-bromophenyl)(4-methoxyphenyl)methyl]-L-leucine.

Step 4: N^2-[(4-bromophenyl)(4-methoxyphenyl)methyl]-N^1-(cyanomethyl)-L-leucinamide

A mixture of *N*-[(4-bromophenyl)(4-methoxyphenyl)methyl]-L-leucine (149 mg, 0.37 mmol) and aminoacetonitrile hydrochloride (87 mg, 0.73 mmol) was dissolved in 5 mL of dimethylformamide. HATU (153 mg, 0.403 mmol) was added in one portion followed by triethylamine (0.18 mL, 1.31 mmol). The mixture was stirred overnight, then poured into pH 4 phosphate buffer (40 mL) and extracted with ethyl acetate (50 mL). The organic phase was washed with three 50-mL portions of water, dried over sodium sulfate and concentrated under reduced pressure. Purification over silica gel (30% ethyl acetate/hexanes) provided N^2-[(4-bromophenyl)(4-methoxyphenyl)methyl]-N^1-(cyanomethyl)-L-leucinamide.

MS (+ESI): 443.9 [M+1].

CATHEPSIN BINDING

Each of Examples 1 through 26 was submitted to the following procedure: within the context of a computer-generated model, the compound was energy-minimized in the active site of Cathepsin K. The computer-generated model of Cathepsin K was based on the crystal structures of the Protein Databank entry 1MEM, to which hydrogens had been added, but no energy minimization of non-hydrogens had been performed at the start of the energy minimization (therefore protein sidechains retained the geometry of the Xray crystal structure). The binding orientation of the compound was determined based on the following assumptions: 1) that a covalent bond is formed in S1 between the electrophilic carbon labeled "1" in the compound. In conjunction with the chemical formula for the invention, this determines the molecular fragments corresponding to R_1 and R_2; 2) for the fragment of the compound corresponding to the formula of the invention, the amine hydrogen forms a hydrogen bond with the oxygen of Gly66, therefore having a distance of less than 4 Å between these two atoms; 3) The fragments of the compound corresponding to R_2 and R_3 were placed in the cathepsin subsites S_2 and S_3, respectively, according to the labeled carbon atoms on the chemical structure for the compound. For example, the carbon labeled "2" in the structure was located in S2 and the carbon labeled "3" was located in S3, such that the distances to cathepsin Cα's in Table 1 were within an angstrom of those given in Table 1. Although these placements were done manually and approximately, they were placed intentionally to lead to favorable interactions. For compounds synthesized as racemic mixtures, the enantiomer corresponding to the chemical formula of the invention was used for the calculation. The energy minimization was carried out using the software MacroModel with the MMFFs force field. All atoms of the compound were allowed to move, but for Cathepsin K only protein sidechains having an atom within 6 Å of the compound were allowed to move; in this case the whole sidechain could move. Default parameters for the energy minimization were chosen, with a continuum solvent option corresponding to water. The results of the energy minimization for the compound was favorable: there were no bad steric interactions between the ligand and the active site of Cathepsin K, and favorable interactions between the active site and the ligand (lipophilic interactions and hydrogen bonding) are confirmed by the distances given in Table 1. Table 1 gives distances taken from the energy-minimized ligand-enzyme complexes formed by the compounds and Cathepsin K as described above. The column labeled "Gly66 Hbond" gives the distance of the hydrogen bond formed between the amine hydrogen of the compound and the oxygen of Gly66. The three columns under R2 in Table 1 are headed by a Cα label corresponding to a Cα in the cathepsin; these columns give the distance between the indicated Cα in the cathepsin and the carbon labeled "2" in the structure for that compound. Similarly, the two columns under "R3" in Table 1 give the distance between the indicated Cα in the cathepsin and the carbon labeled "3" in the structure for that compound. The covalent bond made between cysteine sulfur of residue 25 in the cathepsin and the electrophilic carbon labeled "1" in the structure for that compound ensured distance of < 5 Å of the carbon labeled "1" in the structure for that compound. Thus Examples 1 through 26 meet the distance criteria described herein.

Table 1. Distances (in Å) from atoms in Cathepsin K to atoms in Examples 1 to 26. See text.

Example		R2			R3	
	Gly66 Hbond	$C\alpha_{26}$	$C\alpha_{68}$	$C\alpha_{134}$	$C\alpha_{66}$	$C\alpha_{60}$
1	2.4	6.4	7.7	5.5	4.5	6.1
2	2.5	6.4	7.7	5.5	4.4	5.9
3	2.5	6.5	7.7	5.5	4.8	6.3
4	2.6	6.4	7.7	5.6	4.8	6.3
5	2.6	6.4	7.7	5.4	4.8	6.3
6	2.5	6.4	7.7	5.5	4.7	6.3
7	2.5	6.4	7.7	5.5	4.6	6.1
8	2.5	6.5	7.6	5.4	4.7	6.3
9	2.4	6.5	7.8	5.3	4.6	6.2
10	2.5	6.8	8.0	5.4	4.6	6.1
11	2.6	6.6	7.7	5.4	4.8	6.3
12	2.6	6.2	7.7	5.6	4.8	6.5
13	2.7	6.7	7.9	5.6	5.0	6.5
14	2.5	6.5	7.7	5.5	4.5	6.0
15	2.5	6.5	7.7	5.4	4.7	6.3
16	2.4	6.5	7.5	5.4	4.7	6.2
17	2.4	6.6	7.5	5.4	4.7	6.2
18	2.4	6.4	7.5	5.4	4.7	6.2
19	2.5	6.7	7.5	5.4	4.8	6.2
20	2.3	6.4	7.5	5.5	4.5	6.1
21	2.4	6.4	7.6	5.4	4.6	6.2
22	2.5	6.5	7.6	5.5	4.7	6.2
23	2.4	6.4	7.6	5.4	4.7	6.3
24	2.0	6.7	7.3	5.4	4.6	6.0
25	2.4	6.5	7.6	5.4	4.6	6.2
26	2.4	6.4	7.7	5.5	4.8	6.4

PURIFIED ENZYME ASSAYS

The compounds disclosed in the present application exhibited activity in the following assays. In addition, the compounds disclosed in the present application have an enhanced pharmacological profile relative to previously disclosed compounds.

Cathepsin K Assay

Serial dilutions (1/3) from 500 μM down to 0.0085 μM of test compounds were prepared in dimethyl sulfoxide (DMSO). Then 2 μL of DMSO from each dilution were added to 50 μL of assay buffer (MES, 50 mM (pH 5.5); EDTA, 2.5 mM; DTT, 2.5 mM and 10% DMSO) and 25 μL of human cathepsin K (0.4 nM) in assay buffer solution. The assay solutions were mixed for 5-10 seconds on a shaker plate and incubated for 15 minutes at room temperature. Z-Leu-Arg-AMC (8 μM) in 25 μL of assay buffer was added to the assay solutions. Hydrolysis of the coumarin leaving group (AMC) was followed by spectrofluorometry (Exλ =355 nm; Emλ = 460 nm) for 10 minutes. Percent of inhibition were calculated by fitting experimental values to standard mathematical model for dose response curve.

Cathepsin L Assay

Serial dilutions (1/3) from 500 μM down to 0.0085 μM of test compounds were prepared in dimethyl sulfoxide (DMSO). Then 2 μL of DMSO from each dilution were added to 50 μL of assay buffer (MES, 50 mM (pH 5.5); EDTA, 2.5 mM; DTT, 2.5 mM and 10% DMSO) and 25 μL of human cathepsin L (0.5 nM) in assay buffer solution. The assay solutions were mixed for 5-10 seconds on a shaker plate and incubated for 15 minutes at room temperature. Z-Leu-Arg-AMC (8 μM) in 25 μL of assay buffer was added to the assay solutions. Hydrolysis of the coumarin leaving group (AMC) was followed by spectrofluorometry (Exλ =355 nm; Emλ = 460 nm) for 10 minutes. Percent of inhibition were calculated by fitting experimental values to standard mathematical model for dose response curve.

Cathepsin B Assay

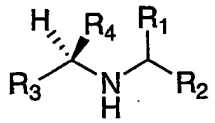
Serial dilutions (1/3) from 500 μM down to 0.0085 μM of test compounds were prepared in dimethyl sulfoxide (DMSO). Then 2 μL of DMSO from each dilution were added to 50 μL of assay buffer (MES, 50 mM (pH 5.5); EDTA, 2.5 mM; DTT, 2.5 mM and 10% DMSO) and 25 μL of human cathepsin B (4.0 nM) in assay buffer solution. The assay solutions were mixed for 5-10 seconds on a shaker plate and incubated for 15 minutes at room temperature. Z-Leu-Arg-AMC (8 μM) in 25 μL of assay buffer was added to the assay solutions. Hydrolysis of the coumarin leaving group (AMC) was followed by spectrofluorometry (Exλ =355 nm; Emλ = 460 nm) for 10 minutes. Percent of inhibition were calculated by fitting experimental values to standard mathematical model for dose response curve.

Cathepsin S Assay

Serial dilutions (1/3) from 500 μM down to 0.0085 μM of test compounds were prepared in dimethyl sulfoxide (DMSO). Then 2 μL of DMSO from each dilution were added to 50 μL of assay buffer (MES, 50 mM (pH 5.5); EDTA, 2.5 mM; DTT, 2.5 mM and 10% DMSO) and 25 μL of human cathepsin S (20 nM) in assay buffer solution. The assay solutions were mixed for 5-10 seconds on a shaker plate and incubated for 15 minutes at room temperature. Z-Leu-Arg-AMC (8 μM) in 25 μL of assay buffer was added to the assay solutions. Hydrolysis of the coumarin leaving group (AMC) was followed by spectrofluorometry (Exλ =355 nm; Emλ = 460 nm) for 10 minutes. Percent of inhibition were calculated by fitting experimental values to standard mathematical model for dose response curve.

WHAT IS CLAIMED IS:

1. A compound having the chemical formula:



and having no more than 70 nonhydrogen atoms each independently selected from C, O, N, S, P, F, Cl, Br or I;

wherein R_4 is a non-hydrogen electron-withdrawing substituent such that, together with R_1, R_2 and R_3, the basicity of the nitrogen is lowered to less than a pKa of 6;

wherein a molecule of the compound is interacting with a cathepsin such that the CH-NH region in the chemical formula is interacting favorably with the cathepsin between S2 and S3, R_1 interacts favorably with S1 but not S3 of the active site of the cathepsin active site, R_2 interacts favorably with S2 but not S3 of the cathepsin active site, and R_3 interacts favorably with S3 but not S2 or S1 of the cathepsin active site.

2. The compound of Claim 1 wherein the cathepsin is selected from cathepsin B, F, H, K, L, L_2, O, S, W or Z.

3. The compound of Claim 2 wherein the cathepsin is selected from cathepsin K, L, S or B.

4. The compound of Claim 1, wherein R_4 is not interacting favorably with subsites S_2, S_3 and S_1, respectively, of a cathepsin active site.

5. The compound of Claim 1, wherein R_2 has at least one carbon or sulfur atom which simultaneously fulfills the following three distance critieria: it is within 7 Å of $C\alpha_{26}$, and it is within 8.5 Å of $C\alpha_{68}$ and it is within 7 Å of $C\alpha_{134}$ of a cathepsin.

6. The compound of Claim 1, wherein R_3 has at least one carbon or sulfur atom which simultaneously fulfills the following two distance critieria: it is within 5.5 Å of $C\alpha_{66}$, and it is within 7 Å of $C\alpha_{60}$ of a cathepsin.

7. The compound of Claim 1, wherein the nitrogen has a pKa of less than 6 and makes a hydrogen bond with the cathepsin amide carbonyl of glycine 66 of a cathepsin.

8. The compound of Claim 1, wherein R_2 comprises nonpolar regions.

9. The compound of Claim 1, wherein R_2 comprises lipophilic regions.

10. The compound of Claim 1, wherein R_3 comprises nonpolar regions.

11. The compound of Claim 1, wherein R_3 comprises lipophilic regions.

12. The compound of Claim 1, wherein the pKa of the nitrogen of the secondary amine shown in claim 1 is <5 in an aqueous medium.

13. The compound of Claim 1, wherein R_4 is a group selected from $-CF_3$, $-CHF_2$, $-CH_2F$, $-CF_2R_5$, and $-CHFR_5$, wherein R_5 is C_{1-6} alkyl, aryl or heteroaryl optionally substituted with 1 to 4 substituents selected from halo, C_{1-3} alkyl, C_{1-3} alkoxy, hydroxy, hydroxyalkyl, keto, cyano, heterocyclyl, C_{3-8} cycloalkyl, SO_mC_{1-3} alkyl, NH_2, NO_2 or $O(C=O)C_{1-3}$ alkyl; and m is an integer from zero to two.

14. The compound of Claim 1, wherein the R_1 comprises a region that stably fits into subsite S_1 of a cathepsin active site, having at least one carbon atom within 5 Å of $C\alpha_{25}$ of a cathepsin.

15. The compound of Claim 14 wherein the cathepsin is selected from cathepsin B, F, H, K, L, L_2, O, S, W or Z.

16. The compound of Claim 14, wherein the compound forms a covalent bond with the sulfur of cysteine 25 of a cathepsin.

17. The compound of Claim 14, wherein R_1 is non-immunogenic.

18. The compound of Claim 14, wherein the compound binds to the active site of a cathepsin with an IC_{50} of less than 10 micromolar in a purified enzyme assay.

19. The compound of Claim 14, wherein a covalent bond is made to an electrophilic carbonyl carbon of the compound.

20. The compound of Claim 1, wherein no covalent bond is formed between the compound and a cathepsin.

21. A pharmaceutical composition comprising a compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier.

22. Use of a compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for inhibiting cathepsin activity, or treating or preventing cathepsin dependent conditions in a mammal, or inhibiting bone loss or reducing bone loss in a mammal, or treating or preventing osteoporosis in a mammal, or treating or preventing rheumatoid arthritis condition in a mammal, or treating or preventing progression of osteoarthritis in a mammal, or treating cancer in a mammal.

23. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof for use in a medicament therapy.

24. A method of treating or preventing cathepsin dependent conditions in a mammal, or inhibiting bone loss or reducing bone loss in a mammal, or treating or preventing osteoporosis in a mammal, or treating or preventing rheumatoid arthritis condition in a mammal, or treating or preventing progression of osteoarthritis in a mammal, or treating cancer in a mammal comprising administering to a mammal a therapeutically effective amount of a compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof.

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                85                  90                  95
Ser Cys Lys Tyr Asn Pro Lys Tyr Ser Val Ala Asn Asp Thr Gly Phe
            100                 105                 110
Val Asp Ile Pro Lys Gln Glu Lys Ala Leu Met Lys Ala Val Ala Thr
        115                 120                 125
Val Gly Pro Ile Ser Val Ala Ile Asp Ala Gly His Glu Ser Phe Leu
    130                 135                 140
Phe Tyr Lys Glu Gly Ile Tyr Phe Glu Pro Asp Cys Ser Ser Glu Asp
145                 150                 155                 160
Met Asp His Gly Val Leu Val Val Gly Tyr Gly Phe Glu Ser Thr Glu
                165                 170                 175
Ser Asp Asn Asn Lys Tyr Trp Leu Val Lys Asn Ser Trp Gly Glu Glu
            180                 185                 190
Trp Gly Met Gly Gly Tyr Val Lys Met Ala Lys Asp Arg Arg Asn His
        195                 200                 205
Cys Gly Ile Ala Ser Ala Ala Ser Tyr Pro Thr Val
    210                 215                 220
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<210> 6
<211> 214
<212> PRT
<213> HOMO SAPIENS

<400> 6

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 1               5                  10                  15
Arg Asn Gln Gln Met Cys Gly Gly Cys Trp Ala Phe Ser Val Val Gly
            20                  25                  30
Ala Val Glu Ser Ala Tyr Ala Ile Lys Gly Lys Pro Leu Glu Asp Leu
        35                  40                  45
Ser Val Gln Gln Val Ile Asp Cys Ser Tyr Asn Asn Tyr Gly Cys Asn
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    50                  55                  60
Gly Gly Ser Thr Leu Asn Ala Leu Asn Trp Leu Asn Lys Met Gln Val
65                  70                  75                  80
Lys Leu Val Lys Asp Ser Glu Tyr Pro Phe Lys Ala Gln Asn Gly Leu
                85                  90                  95
Cys His Tyr Phe Ser Gly Ser His Ser Gly Phe Ser Ile Lys Gly Tyr
            100                 105                 110
Ser Ala Tyr Asp Phe Ser Asp Gln Glu Asp Glu Met Ala Lys Ala Leu
        115                 120                 125
Leu Thr Phe Gly Pro Leu Val Val Ile Val Asp Ala Val Ser Trp Gln
    130                 135                 140
Asp Tyr Leu Gly Gly Ile Ile Gln His His Cys Ser Ser Gly Glu Ala
145                 150                 155                 160
Asn His Ala Val Leu Ile Thr Gly Phe Asp Lys Thr Gly Ser Thr Pro
                165                 170                 175
Tyr Trp Ile Val Arg Asn Ser Trp Gly Ser Ser Trp Gly Val Asp Gly
            180                 185                 190
Tyr Ala His Val Lys Met Gly Ser Asn Val Cys Gly Ile Ala Asp Ser
        195                 200                 205
Val Ser Ser Ile Phe Val
    210
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<210> 7
<211> 217
<212> PRT
<213> HOMO SAPIENS

<400> 7

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Leu Pro Asp Ser Val Asp Trp Arg Glu Lys Gly Cys Val Thr Glu Val
 1               5                  10                  15
Lys Tyr Gln Gly Ser Cys Gly Ala Cys Trp Ala Phe Ser Ala Val Gly
            20                  25                  30
Ala Leu Glu Ala Gln Leu Lys Leu Lys Thr Gly Lys Leu Val Thr Leu
        35                  40                  45
Ser Ala Gln Asn Leu Val Asp Cys Ser Thr Glu Lys Tyr Gly Asn Lys
    50                  55                  60
Gly Cys Asn Gly Gly Phe Met Thr Thr Ala Phe Gln Tyr Ile Ile Asp
65                  70                  75                  80
Asn Lys Gly Ile Asp Ser Asp Ala Ser Tyr Pro Tyr Lys Ala Met Asp
                85                  90                  95
Gln Lys Cys Gln Tyr Asp Ser Lys Tyr Arg Ala Ala Thr Cys Ser Lys
            100                 105                 110
Tyr Thr Glu Leu Pro Tyr Gly Arg Glu Asp Val Leu Lys Glu Ala Val
        115                 120                 125
Ala Asn Lys Gly Pro Val Ser Val Gly Val Asp Ala Arg His Pro Ser
    130                 135                 140
Phe Phe Leu Tyr Arg Ser Gly Val Tyr Tyr Glu Pro Ser Cys Thr Gln
145                 150                 155                 160
Asn Val Asn His Gly Val Leu Val Val Gly Tyr Gly Asp Leu Asn Gly
                165                 170                 175
Lys Glu Tyr Trp Leu Val Lys Asn Ser Trp Gly His Asn Phe Gly Glu
            180                 185                 190
Glu Gly Tyr Ile Arg Met Ala Arg Asn Lys Gly Asn His Cys Gly Ile
        195                 200                 205
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Ala Ser Phe Pro Ser Tyr Pro Glu Ile
    210                 215
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<210> 8
<211> 249
<212> PRT
<213> HOMO SAPIENS

<400> 8

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Val Pro Phe Ser Cys Asp Trp Arg Lys Val Ala Gly Ala Ile Ser Pro
 1               5                  10                  15
Ile Lys Asp Gln Lys Asn Cys Asn Cys Cys Trp Ala Met Ala Ala Ala
            20                  25                  30
Gly Asn Ile Glu Thr Leu Trp Arg Ile Ser Phe Trp Asp Phe Val Asp
        35                  40                  45
Val Ser Val His Glu Leu Leu Asp Cys Gly Arg Cys Gly Asp Gly Cys
    50                  55                  60
His Gly Gly Phe Val Trp Asp Ala Phe Ile Thr Val Leu Asn Asn Ser
65                  70                  75                  80
Gly Leu Ala Ser Glu Lys Asp Tyr Pro Phe Gln Gly Lys Val Arg Ala
                85                  90                  95
His Arg Cys His Pro Lys Lys Tyr Gln Lys Val Ala Trp Ile Gln Asp
            100                 105                 110
Phe Ile Met Leu Gln Asn Asn Glu His Arg Ile Ala Gln Tyr Leu Ala
        115                 120                 125
Thr Tyr Gly Pro Ile Thr Val Thr Ile Asn Met Lys Pro Leu Gln Leu
    130                 135                 140
Tyr Arg Lys Gly Val Ile Lys Ala Thr Pro Thr Thr Cys Asp Pro Gln
145                 150                 155                 160
Leu Val Asp His Ser Val Leu Leu Val Gly Phe Gly Ser Val Lys Ser
                165                 170                 175
Glu Glu Gly Ile Trp Ala Glu Thr Val Ser Ser Gln Ser Gln Pro Gln
            180                 185                 190
Pro Pro His Pro Thr Pro Tyr Trp Ile Leu Lys Asn Ser Trp Gly Ala
        195                 200                 205
Gln Trp Gly Glu Lys Gly Tyr Phe Arg Leu His Arg Gly Ser Asn Thr
    210                 215                 220
Cys Gly Ile Thr Lys Phe Pro Leu Thr Ala Arg Val Gln Lys Pro Asp
225                 230                 235                 240
Met Lys Pro Arg Val Ser Cys Pro Pro
                245
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<211> 242
<212> PRT
<213> HOMO SAPIENS

<400> 9

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 1               5                  10                  15
Ser Ile Thr Arg Asn Gln His Ile Pro Gln Tyr Cys Gly Ser Cys Trp
            20                  25                  30
Ala His Ala Ser Thr Ser Ala Met Ala Asp Arg Ile Asn Ile Lys Arg
```

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        35                  40                  45
Lys Gly Ala Trp Pro Ser Thr Leu Leu Ser Val Gln Asn Val Ile Asp
    50                  55                  60
Cys Gly Asn Ala Gly Ser Cys Glu Gly Gly Asn Asp Leu Ser Val Trp
65                  70                  75                  80
Asp Tyr Ala His Gln His Gly Ile Pro Asp Glu Thr Cys Asn Asn Tyr
                85                  90                  95
Gln Ala Lys Asp Gln Glu Cys Asp Lys Phe Asn Gln Cys Gly Thr Cys
            100                 105                 110
Asn Glu Phe Lys Glu Cys His Ala Ile Arg Asn Tyr Thr Leu Trp Arg
        115                 120                 125
Val Gly Asp Tyr Gly Ser Leu Ser Gly Arg Glu Lys Met Met Ala Glu
    130                 135                 140
Ile Tyr Ala Asn Gly Pro Ile Ser Cys Gly Ile Met Ala Thr Glu Arg
145                 150                 155                 160
Leu Ala Asn Tyr Thr Gly Gly Ile Tyr Ala Glu Tyr Gln Asp Thr Thr
                165                 170                 175
Tyr Ile Asn His Val Val Ser Val Ala Gly Trp Gly Ile Ser Asp Gly
            180                 185                 190
Thr Glu Tyr Trp Ile Val Arg Asn Ser Trp Gly Glu Pro Trp Gly Glu
        195                 200                 205
Arg Gly Trp Leu Arg Ile Val Thr Ser Thr Tyr Lys Asp Gly Lys Gly
    210                 215                 220
Ala Arg Tyr Asn Leu Ala Ile Glu Glu His Cys Thr Phe Gly Asp Pro
225                 230                 235                 240
Ile Val
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<210> 10
<211> 221
<212> PRT
<213> HOMO SAPIENS

<400> 10

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Leu Pro Lys Ser Val Asp Trp Arg Lys Lys Gly Tyr Val Thr Pro Val
 1               5                  10                  15
Lys Asn Gln Lys Gln Cys Gly Ser Cys Trp Ala Phe Ser Ala Thr Gly
            20                  25                  30
Ala Leu Glu Gly Gln Met Phe Arg Lys Thr Gly Lys Leu Val Ser Leu
        35                  40                  45
Ser Glu Gln Asn Leu Val Asp Cys Ser Arg Pro Gln Gly Asn Gln Gly
    50                  55                  60
Cys Asn Gly Gly Phe Met Ala Arg Ala Phe Gln Tyr Val Lys Glu Asn
65                  70                  75                  80
Gly Gly Leu Asp Ser Glu Glu Ser Tyr Pro Tyr Val Ala Val Asp Glu

                85                  90                  95
Ile Cys Lys Tyr Arg Pro Glu Asn Ser Val Ala Asn Asp Thr Gly Phe
            100                 105                 110
Thr Val Val Ala Pro Gly Lys Glu Lys Ala Leu Met Lys Ala Val Ala
        115                 120                 125
Thr Val Gly Pro Ile Ser Val Ala Met Asp Ala Gly His Ser Ser Phe
    130                 135                 140
Gln Phe Tyr Lys Ser Gly Ile Tyr Phe Glu Pro Asp Cys Ser Ser Lys
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145                 150                 155                 160
Asn Leu Asp His Gly Val Leu Val Val Gly Tyr Gly Phe Glu Gly Ala
                165                 170                 175
Asn Ser Asn Asn Ser Lys Tyr Trp Leu Val Lys Asn Ser Trp Gly Pro
            180                 185                 190
Glu Trp Gly Ser Asn Gly Tyr Val Lys Ile Ala Lys Asp Lys Asn Asn
        195                 200                 205
His Cys Gly Ile Ala Thr Ala Ala Ser Tyr Pro Asn Val
    210                 215                 220
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<210> 11
<211> 237
<212> PRT
<213> PLASMODIUM FALCIPARUM

<400> 11

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Val Pro Glu Ile Leu Asp Tyr Arg Glu Lys Gly Ile Val His Glu Pro
 1               5                  10                  15
Lys Asp Gln Gly Leu Cys Gly Ser Cys Trp Ala Phe Ala Ser Val Gly
            20                  25                  30
Asn Ile Glu Ser Val Phe Ala Lys Lys Asn Lys Asn Ile Leu Ser Phe
        35                  40                  45
Ser Glu Gln Glu Val Val Asp Cys Ser Lys Asp Asn Phe Gly Cys Asp
    50                  55                  60
Gly Gly His Pro Phe Tyr Ser Phe Leu Tyr Val Leu Gln Asn Glu Leu
65                  70                  75                  80
Cys Leu Gly Asp Glu Tyr Lys Tyr Lys Ala Lys Asp Asp Met Phe Cys
                85                  90                  95
Leu Asn Tyr Arg Cys Lys Arg Lys Val Ser Leu Ser Ser Ile Gly Ala
            100                 105                 110
Val Lys Glu Asn Gln Leu Ile Leu Ala Leu Asn Glu Val Gly Pro Leu
        115                 120                 125
Ser Val Asn Val Gly Val Asn Asn Asp Phe Val Ala Tyr Ser Glu Gly
    130                 135                 140
Val Tyr Asn Gly Thr Cys Ser Glu Glu Leu Asn His Ser Val Leu Leu
145                 150                 155                 160
Val Gly Tyr Gly Gln Val Glu Lys Thr Lys Leu Asn Tyr Asn Asn Lys
                165                 170                 175
Ile Gln Thr Tyr Asn Thr Lys Glu Asn Ser Asn Gln Pro Asp Asp Asn
            180                 185                 190
Ile Ile Tyr Tyr Trp Ile Ile Lys Asn Ser Trp Ser Lys Lys Trp Gly
        195                 200                 205
Glu Asn Gly Phe Met Arg Leu Ser Arg Asn Lys Asn Gly Asp Asn Val
    210                 215                 220
Phe Cys Gly Ile Gly Glu Glu Val Phe Tyr Pro Ile Leu
225                 230                 235
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<210> 12
<211> 222
<212> PRT
<213> PLASMODIUM FALCIPARUM

<400> 12

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Asp Arg Ile Ala Tyr Asp Trp Arg Leu His Gly Gly Val Thr Pro Val
 1               5                  10                  15
Lys Asp Gln Ala Leu Cys Gly Ser Cys Trp Ala Phe Ser Ser Val Gly
            20                  25                  30
Ser Val Glu Ser Gln Tyr Ala Ile Arg Lys Leu Phe Leu Phe Ser Glu
        35                  40                  45
Gln Glu Leu Val Asp Cys Ser Val Lys Asn Asn Gly Cys Tyr Gly Gly
    50                  55                  60
Tyr Ile Thr Asn Ala Phe Asp Asp Met Ile Asp Leu Gly Gly Leu Cys
65                  70                  75                  80

Ser Gln Asp Asp Tyr Pro Tyr Val Ser Asn Leu Pro Glu Thr Cys Asn
                85                  90                  95
Leu Lys Arg Cys Asn Glu Arg Tyr Thr Ile Lys Ser Tyr Val Ser Ile
            100                 105                 110
Pro Asp Asp Lys Phe Lys Glu Ala Leu Arg Tyr Leu Gly Pro Ile Ser
        115                 120                 125
Ile Ser Ile Ala Ala Ser Asp Asp Phe Ala Phe Tyr Arg Gly Gly Phe
    130                 135                 140
Tyr Asp Gly Glu Cys Gly Ala Ala Pro Asn His Ala Val Ile Leu Val
145                 150                 155                 160
Gly Tyr Gly Met Lys Asp Ile Tyr Asn Glu Asp Thr Gly Arg Met Glu
                165                 170                 175
Lys Phe Tyr Tyr Tyr Ile Ile Lys Asn Ser Trp Gly Ser Asp Trp Gly
            180                 185                 190
Glu Gly Gly Tyr Ile Asn Leu Glu Thr Asp Glu Asn Gly Tyr Lys Lys
        195                 200                 205
Thr Cys Ser Ile Gly Thr Glu Ala Tyr Val Pro Leu Leu Glu
    210                 215                 220


<210> 13
<211> 224
<212> PRT
<213> PLASMODIUM FALCIPARUM

<400> 13
Asp His Ala Ala Tyr Asp Trp Arg Leu His Ser Gly Val Thr Pro Val
 1               5                  10                  15
Lys Asp Gln Lys Asn Cys Gly Ser Cys Trp Ala Phe Ser Ser Ile Gly
            20                  25                  30
Ser Val Glu Ser Gln Tyr Ala Ile Arg Lys Asn Lys Leu Ile Thr Leu
        35                  40                  45
Ser Glu Gln Glu Leu Val Asp Cys Ser Phe Lys Asn Tyr Gly Cys Asn
    50                  55                  60
Gly Gly Leu Ile Asn Asn Ala Phe Glu Asp Met Ile Glu Leu Gly Gly
65                  70                  75                  80
Ile Cys Pro Asp Gly Asp Tyr Pro Tyr Val Ser Asp Ala Pro Asn Leu
                85                  90                  95
Cys Asn Ile Asp Arg Cys Thr Glu Lys Tyr Gly Ile Lys Asn Tyr Leu
            100                 105                 110
Ser Val Pro Asp Asn Lys Leu Lys Glu Ala Leu Arg Phe Leu Gly Pro
        115                 120                 125
Ile Ser Ile Ser Val Ala Val Ser Asp Asp Phe Ala Phe Tyr Lys Glu
    130                 135                 140
```

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Gly Ile Phe Asp Gly Glu Cys Gly Asp Glu Leu Asn His Ala Val Met
145                 150                 155                 160
Leu Val Gly Phe Gly Met Lys Glu Ile Val Asn Pro Leu Thr Lys Lys
                165                 170                 175
Gly Glu Lys His Tyr Tyr Tyr Ile Ile Lys Asn Ser Trp Gly Gln Gln
            180                 185                 190
Trp Gly Glu Arg Gly Phe Ile Asn Ile Glu Thr Asp Glu Ser Gly Leu
        195                 200                 205
Met Arg Lys Cys Gly Leu Gly Thr Asp Ala Phe Ile Pro Leu Ile Glu
    210                 215                 220
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2004/001577

A. CLASSIFICATION OF SUBJECT MATTER
IPC 07 C07C 255/29, C07C 317/22, C07C 317/40, C07D 241/04, C07D 333/10

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC 07 C07C, C07D

Electronic data base consulted during the international search (name of data base, and, where practicable, search terms used) keywords: "cysteine", "cathepsin", "secondary adj alkylamine", "protease", Canadian Patent Database; PLUSPAT, STN-CAPLUS, ACS PUB, BIO MED CENTRAL, WEST, SCIRUS; DELPHION, HighWire, EPO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL. MED. CHEM. (DE LOMBAERT, ET AL) Vol. 43. No. 3, 2000, Pages 488-504 "Potent and Selective Non-peptide Inhibitors of Endothelin-Converting Enzyme-1 with Sustained Duration of Action" 3-dibenzofuranproponoic acid α-[[(1R)-3-(1-naphthalenyl)-1-phosphonopropyl]amino] trisodum salt	1-13, 15-24
X	US A 4,721,726 (BERGER, J.G) 9 September 1986 B-alanyl, N-[3-(1,1'-biphenyl)-4-yl—[1-[[1,1'-biphenyl]-4-ylmethyl]-2-oxo-2-(2-phenoxyethoxy)ethyl]-L-alanyl]-2-methyl (S); Col 26 lines 9, 24, 27, 39 and 54; Col 28 lines 42, 53 and 65; Compounds in Table 1, Cols 9 and 10, Examples	1-13, 15-24

Further documents are listed in the continuation of Box C [X].

Patent family members are listed in annex [X].

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international-type search	Date of mailing of the international-type search report
25 November 2004 (25-11-2004)	14 January 2005 (14-01-2005)
Name and mailing address of the ISA/ *Commissioner of Patents* *Canadian Patent Office - PCT* *Ottawa/Gatineau K1A 0C9* Facsimile No. 1-819-953-9358	Authorized officer Okemona Oke (819) 956-4108

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2004/001577

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US A 4,748,160 (BENNION, ET AL) 31 May 1988 Benzenebutanoic acid, α-[[2-(1-[2-(1,1-dimethylethoxy)-2-oxoethyl] hydrazino]-1-methyl-2-oxoethyl]amino]-ethyl ester; Col 8 line 23, 25 and 29; Examples 5 and 6	1-13, 15-24
A	US A 6,518,267 (BONDINELL, ET AL) 11 February 2003 see whole document	1
A	US A 20030105099 (GRAUPE, ET AL) 5 June 2003 see whole document	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2004/001577

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claims Nos. : 24
because they relate to subject matter not required to be searched by this Authority; namely:

Although claim 24 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition

2. [X] Claims Nos.: 1-23
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

(see supplementary sheet)

3. [ ] Claims Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III **Observation where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos. :

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest.
[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2004/001577

Continuation of **Box No. II**:

Present claims 1-23 relate to compounds which are defined by reference to a desired feature, function or property, namely, their interaction or binding with specific regions of a cathepsin enzyme (No claim comprise the essential features of the invention wherein all of R^1, R^2, R^3 and R^4 are defined in concrete and explicit terms). This is further complicated by the fact that it is not clear if claims 1-23 are directed to a substrate-enzyme complex comprising the alkylamine compound-cathepsin complex. For instance, claims 1-3, 5-19 and 21-23 (part) appear to be directed to a compound of the formula depicted in claim 1, a molecule of which "is interacting with a cathepsin" whereas claims 4 and 20 appear to be directed to a compound of formula depicted in claim 1, a molecule of which "is not interacting favorably with a cathepsin active site" (claim 4) or "no covalent bond is formed between the compound and cathepsin" (claim 20). The claims cover all conceivable compounds (the magnitude is infinite) having the above features whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. A preliminary search based on the structure depicted in claim 1 could not be completed due to the volume of results. In the present case, the claims so lack support and the application so lack enabling disclosure commensurate with the scope claimed that a meaningful and complete search over the entire range of the claimed subject matter is impossible. Independent of the above reasoning, the claims, by attempting to define the compounds by reference to a desired effect, function or result, also lack clarity (Article 6 PCT), as such a meaningful and complete search over the entire range of the claimed subject matter is impossible. Accordingly, a search has been limited to the closely structurally related compounds and to specific compounds of the invention as taught and fully supported in the Examples of the description (pages 43-87 & PCT Guideline 9.21).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/CA2004/001577

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 4748160 A	31.05.1988	JP61143349	A2	01-07-1986
		GB8505798	A0	11-04-1985
		EP01833398	A1	04-06-1986
		DE3569368	C0	18-05-1989
US 4721726 A	26-01-1988	ZA8308721	A	27-06-1984
		US4906615	B1	06-03-1990
		US4610816	B1	09-09-1986
		SG0074590	A	23-11-1990
		SG0070787	A	19-02-1988
		PT0077699	A	01-12-1983
		NZ0201001	A	21-02-1986
		NZ0206362	A	29-11-1988
		NO0834294	A	28-05-1984
		NO069172	B	10-02-1992
		MY0075987	A	31-12-1987
		KE0003759	A	02-10-1987
		JP59031744	A2	20-02-1984
		JP58032851	A2	25-02-1983
		JP03079339	B4	18-12-1991
		JP03022870	B4	27-03-1991
		IL0070309	A1	30-11-1988
		IL0066079	A1	31-12-1987
		IE0055836	B	30-01-1991
		IE0053315	B	12-10-1988
		EP0103077	A2	21-03-1984
		EP0054862	A1	30-06-1982
		HU0193887	B	28-12-1987
		DK533483	A0	21-11-1983
		DE3376649	C0	23-06-1988
		DE3173075	C0	09-01-1988
		HK0014091	A	08-03-1991
		HK0094287	A	18-12-1987
		CA1304540	AA	30-06-1992
		CA1250091	AA	14-02-1989
		CY0001406	A	22-04-1988
		FI0834280	A0	23-11-1983
		FI0078917	C	10-10-1989
		AU8494782	A1	22-12-1983
		AU2157783	A1	31-05-1981
		AT0034382	E	15-06-1988
US 6518267 A	11-02-2003	WO9959526	A2	25-11-1999
		JP2002515411	T2	28-05-2002
		EP1067894	A2	17-01-2001
US 20030105099 A	05-06-2003	WO02051983	A2	04-07-2002
		EP1383748	A2	05-08-2004
		CA2433520	AA	04-07-2002